ASTMH
AMERICAN SOCIETY OF TROPICAL MEDICINE & HYGIENE
ADVANCING GLOBAL HEALTH SINCE 1903

VOLUME 103 NOVEMBER 2020 NUMBER 5 SUPPLEMENT

2020 ANNUAL MEETING

NOVEMBER 15–19 | *VIRTUAL MEETING*

astmh.org | ajtmh.org | #TropMed20 #IamTropMed

ABSTRACT BOOK

Supplement to
The American Journal of Tropical Medicine and Hygiene

1

CLIMATIC AND ENVIRONMENTAL DETERMINANTS OF THE SPATIAL DISTRIBUTION AND ABUNDANCE OF DISEASE VECTORS, *IXODES SCAPULARIS* AND *AMBLYOMMA AMERICANUM,* BASED ON PASSIVE SURVEILLANCE IN CONNECTICUT

Eliza A. Little, Kirby Stafford, Goudarz Molaei

Connecticut Agricultural Experiment Station, New Haven, CT, United States

Ticks and tick-borne diseases are increasingly becoming a major public health and veterinary concern. Two tick vectors of importance are the blacklegged tick (*Ixodes scapularis*) and the lone star tick (*Amblyomma americanum*). Climatic (e.g. temperature and humidity) and environmental (e.g. habitat type and host availability) conditions determine the distribution and abundance of ticks and hosts which underly tick-borne disease risk. Understanding the ecological conditions underpinning vector populations is critical to making informed and effective recommendations that reduce tick-borne disease risk. Here we developed spatially explicit statistical models using ecological principles to link extensive tick surveillance to mean annual temperature and the wildland-urban interface to determine spatiotemporal patterns of blacklegged and lone star ticks in Connecticut. We found that the submission rate of blacklegged ticks, long endemic in Connecticut, is not associated with mean annual temperature. While the submission rate of lone star ticks, a species recently reported to be established in Connecticut, was positively associated with mean annual temperature. We linked land use metrics derived from the wildland-urban interface with submission rates of both blacklegged and lone star ticks. Blacklegged tick submission rates were higher in towns with a higher proportion of land classified as intermix, defined as areas where greater than 6.17 houses per square kilometer and vegetation mix. Lone star tick submission rates were higher in towns with a lower proportion of very low-density vegetation, defined as fewer than 6.17 houses per square kilometer and over 50% wildland vegetation, and higher mean annual temperature. These findings have implications for tick-borne disease dynamics in the northeastern U.S. as the climate here continues to warm and for land use planning to mitigate tick-borne disease risk.

2

TRACKING THE EMERGENCE OF TICKS AND TICK-BORNE DISEASES IN NEW YORK THROUGH COMMUNITY-ENGAGED TICK SURVEILLANCE

Charles E. Hart, Erin Reynolds, Jahnavi Reddy-Bhaskar, Meghan Hermance, Allen Esterly, Matthew Mahoney, Ana Martinez, Martin Earl

Upstate Medical University, Syracuse, NY, United States

Tick-borne diseases are continually emerging and reemerging in response to the changing geographic range of North American ticks. This includes the northward expansion of both *Amblyomma americanum* and *Ixodes scapularis*. The latter transmits multiple known pathogens of public health concern. The exotic tick *Hemaphysalis longicornis* also has the potential to complicate this dynamic situation further. Herein, we describe our efforts to understand the geographic expansion of ticks and tick-borne diseases through a community-engaged tick surveillance program. Under this program, individuals in New York can send ticks found on their property, gardens, pets, wild animals, or humans to our laboratory. The senders complete an online form providing information on when and where the tick was found. Upon receiving the ticks, we identify their species and screen them for 9 pathogens. Since June 2019 we have received 3766 ticks from New York. This includes 3129 *I. scapularis*, 250 *D. variabilis*, 84 *A. americanum*, 35 non-scapularis *Ixodes* including *I. cookei*, and others, including two *H. longicornis*. The dates of submission show a biphasic occurrence of *I. scapularis* with peaks in June and October. Other species reached peak submission in early summer. *I. scapularis* ticks were determined to be infected with multiple pathogens, including *B. burgdorferi*, which was present in 35% of adult ticks and 10% of nymphs submitted. *Ehrlichia/Anaplasma, Borrelia miyamotoi,* and *B. microti* were also detected. Deer Tick Virus was also detected in five *I. scapularis ticks*. Coinfections were observed in 4% of the *I. scapularis*. This shows that *B. burgdorferi* is the dominant pathogen of *I. scapularis* ticks and that *A. americanum* has now become prevalent in southern counties. *H. longicornis* was detected at a low rate that can be expected to grow over time; its ability to transmit local pathogens remains unknown. Performing this study for the next few years will allow us to understand the geographical expansion of ticks and tick-borne diseases. We will also be able to understand the influence of environmental changes and human behavior on the spread of ticks and tick-borne diseases.

3

PASSIVE TICK SURVEILLANCE, ENVIRONMENTAL FACTORS AND NEIGHBORING EFFECTS AS PREDICTORS OF LYME DISEASE RISK AT FINE SPATIAL SCALES

Maria Pilar Fernandez[1], Donal Bisanzio[2], Richard Reithinger[2], Jennifer White[3], Melissa A. Prusinski[3], Bryon P. Backenson[3], Maria A. Diuk-Wasser[1]

[1]*Columbia University, New York, NY, United States,* [2]*RTI International, Washington DC, DC, United States,* [3]*New York State Department of Health, Albany, NY, United States*

Lyme disease (LD) is the main vector-borne disease in the United States (US), with continued increase in incidence and geographic range. However, little is known about the factors associated with the temporal and spatial dynamics of LD spread. In this study, we develop a two-step dynamic spatio-temporal model of LD spread at a fine spatial scale (US census tract) in New York State (NYS), harnessing unique longitudinal acarological data collected by the NYS Department of Health. Predictors included passive tick surveillance, environmental features, and spatial connectivity derived from human movement data at a US census tract level over the 2000-20014 period. First, we use a spatial-temporal regression to investigate the effect of the predictors accounting for the effects of neighboring census tracts. Second, we use the result of the regression model to build a diffusion model to better understand LD spread dynamics over time. Among the tested zero-inflated negative binomial regression models to evaluate the temporal autocorrelation of LD, a model with a 2-year lag had the best fit to the data and was selected as the final model. We found that the probability of reporting LD cases during the study period decreased with elevation and increased with deciduous forest coverage. The number of LD cases reported per year was positively associated with the percent of the census tract that was considered Wildland-Urban Interface (WUI), with the greatest effect shown for the interface with medium intensity, compared to high and low intensity, developed areas. LD cases also increased with the number of ticks reported per census tract 3 years prior, after comparing different time lags. Neighboring effects were also significant: LD cases in a census tract increased with increasing WUI percentage, percent of deciduous forest, and number of cases reported from the neighboring tract. This model will provide a tool for predicting the spread of LD, utilizing tick and case surveillance data at multiple lags, and predicting the likelihood of disease emergence at census tract to help guide preparedness and intervention strategies.

4

EFFECT OF INCREASED TEMPERATURE ON HOST SELECTION BY THE BROWN DOG TICK

Laura H. Backus, Andrés M. López-Pérez, Janet E. Foley

University of California Davis, Davis, CA, United States

Multiple genetic lineages of the *Rhipicephalus sanguineus* s.l. species complex (known collectively as the brown dog tick) transmit numerous zoonotic pathogens. The brown dog tick is responsible for the recent and ongoing fatal Rocky Mountain spotted fever outbreaks in the western United States and northern Mexico, as well as other rickettsial diseases around the world. While the brown dog tick typically feeds on dogs for all stages of the life cycle, humans may be bitten incidentally. Previous research suggests that increased temperatures may lead to

increased aggression by the tick, and it is hypothesized that this may result in humans being bitten more frequently, leading to increased pathogen transmission. Using two genetic lineages of the brown dog tick (the "temperate" or *Rh. sanguineus* s.s. and "tropical" lineage), we compared host selection between humans and dogs at 23 ºC and 38 ºC for nymph and adult ticks. Experiments were performed using a modified T-maze, with a human on one side and a dog on the other. Human host selection by adult ticks increased by 18.5% (tropical lineage) and 20.5% (temperate lineage) at 38 ºC compared with 23 ºC, and preference for humans compared to dogs increased significantly. Both lineages of nymphs significantly preferred humans over dogs at the higher temperature. These results suggest that human disease risk for Rocky Mountain spotted fever and other diseases may increase during warmer weather as a result of changed tick behavior.

5

TICK SALIVARY FACTORS EXACERBATE THE CLINICAL OUTCOME OF HEARTLAND VIRUS DISEASE

Erin Reynolds[1], Jacob Wooldridge[2], Heather Stevenson[3], Saravanan Thangamani[1]

[1]*SUNY Upstate Medical University, Syracuse, NY, United States,* [2]*Columbia University, New York, NY, United States,* [3]*University of Texas Medical Branch, Galveston, TX, United States*

Heartland virus (HRTV; *Phenuviridae, Phlebovirus*) is a tick-borne disease that was first isolated in 2009 from two patients in Missouri and is transmitted by the Lone Star tick. Since the initial discovery of HRTV over 40 cases have been identified across 10 states. Like the closely related Huaiyangshan banyangvirus, the causative agent of SFTS, HRTV replication appears to occur primarily in the spleen and results in systemic inflammation. In this work, we have utilized an IFN-α/β mouse model to evaluate the splenomegaly in response to HRTV infection that mimics human clinical outcomes. Three-week-old A129 mice were injected with media or HRTV (10^5 FFU), with and without tick salivary gland extract collected from adult Lone Star ticks. At 3- and 8-days post-infection the spleens were collected, and splenomegaly was observed. Spleens of all infected mice exhibited depleted white pulp, extramedullary hematopoiesis (EH), and absence of germinal centers. The average periarteriolar lymphoid sheath (PALS) diameter in infected spleens was slightly decreased compared to uninfected spleens at 3dpi. In the group that received both HRTV and tick SGE, the clinical outcome of HRTV infection was exacerbated compared to HRTV only infection. EH scores and the presence of viral antigens in spleen were significantly higher in the HRTV + SGE group at 3 dpi. Next-generation sequencing of splenic RNA is ongoing to evaluate the cellular and immunological factors contributing to these pathologies.

6

REPTILE HOSTS OF *IXODES SCAPULARIS*: WHAT ROLE DO REPTILES PLAY IN THE EPIDEMIOLOGY OF LYME DISEASE IN THE SOUTHEASTERN US?

Carrie De Jesus, Samantha Wisely, Coleman Sheehy, David Blackburn

University of Florida, Gainesville, FL, United States

In the southeastern US, reptiles are important hosts for immature *Ixodes scapularis* which is a vector for *Borrelia burgdorferi sensu lato*. Previous studies have implicated particular lizard species as playing a role in the enzootic cycle of *Borrelia burgdorferi s.l*. However, only a handful have attempted to address if these species are competent hosts for *B. burgdorferi s.l* in the southeastern US. For a host species to be considered competent they must interact with the vector, maintain pathogen infection, and the host must pass along the infection to the vector. The objective of this investigation was to examine if *I. scapularis* and southeastern reptiles interact thus fulfilling the first criteria of host competency. We examined reptile specimens from the Florida Museum of Natural History Herpetology collection. Eleven species of native Florida lizards were visually inspected for embedded ticks. A total of 6,564 reptile specimens were examined that were collected from 1902 to 2018, from 65/67 Florida counties. *I. scapularis* were present on reptiles from the Panhandle to the southern tip of Florida. We found that 4.51% (296/6,564) of the lizard specimens were infested with ticks, the majority being from northern Florida. On average lizards had an infestation of 2.22 immature *I. scapularis*. *Plestiodon* skinks (9.2 ticks/lizard) and *Ophisaurus* glass lizards (4.6 ticks/lizard) had the highest infestation of immature *I. scapularis* similar to what previous field studies have found. The broad headed skink (*Plestiodon latieps*) had the highest prevalence of ticks 18.9% (110/580 infested lizards/total lizards examined). Previous, laboratory infection studies found that *P. inexpectatus* and *A. carolinensis* can maintain a *B. burgdorferi s.l* infections. In our study we did not find any ticks on *A. carolinensis*, therefore not fulfilling the criteria to be a component host species. This investigation provides insight into host-vector relationships for reptiles in Florida. Further studies should examine prevalence of *B. burgdorferi s.l* in reptilian hosts to further clarify which species are competent.

7

ROLE OF NON-*IXODES* TICKS TRANSMITTING *BABESIA* SPP. IN DOGS IN THE US

Kurayi Mahachi, Breanna Scorza, Julia Poje, Eric Kontowicz, Tyler Baccam, Christine Petersen

University of Iowa, Iowa City, IA, United States

Babesiosis, caused by piroplasmid protozoans of the *Babesia* genus, is a significant tick-borne disease of livestock and companion animals in the United States. Previous studies identified a significantly higher prevalence of babesiosis in both people and companion animals. Toepp et al. and Mahachi et al. demonstrated that there was equal babesiosis in dogs across the US, which did not match with the geographic region of the putative vector, *Ixodes scapularis.* Alternative ticks may vector the transmission of *Babesia* spp. to companion animals in the US. Prior research suggests *Dermacentor (Anocentor) nitens*, *D. albipictus,* and *Haemaphysalis longicornis,* potentially vector equine and canine babesiosis in North America. While *Rhipcephalus sanguineous* potentially vectors *B. canis* and *B. gibsoni.* The definitive vector for *B. duncani* in the US has yet to be recognized. Investigating the role of other potential vectors for *Babesia* spp. in companion animals in the United States is essential for public health prevention. We performed a study to investigate the role of non-*Ixodid* tick transmission of *Babesia* spp. in US hunting dogs. We hypothesize that *R. sanguineous* associated with increased risk for *Babesia* spp. in the US. Collected ticks from six dog kennels in the US were identified at the species level and tested via qPCR for *Babesia* spp. Statistical methods were used to investigate alternative tick vectors for *Babesia* spp., including *B. canis, B. gibsoni,* and *B. duncani.* Based on our dragging areas, we identified 70% *Dermacentor* spp., 15% *Amblyomma americanum,* 9% *R. sanguineous,* and 6% *Ixodes scapularis.* When sampling mice, ratios were similar to those from dragging. Ticks found on dogs were majority *Dermacentor* spp. and *A. americanum*. *Babesia* spp. was identified in *R. sanguineous* and *D. albipictus*. This suggests that *R. sanguineous* may play a role in *Babesia* spp. transmission. Evaluation of *Rhipicephalus sanguineous* as a vector for *Babesia* spp. is essential for understanding spread of these parasites and better ways to prevent it.

8

IMPACT OF GUT MICROBIOME ORGANISM *PARACOCCUS AMINOVORANS* ON *VIBRIO CHOLERAE* VIRULENCE

Denise Chac[1], Kelsey Barasso[2], Meti Debela[3], Stephen Calderwood[3], Edward Ryan[3], Regina LaRocque[3], Taufiq Bhuiyan[4], Jason Harris[3], Firdausi Qadri[4], Wai-Leung Ng[2], **Ana Weil**[1]

[1]*University of Washington, Seattle, WA, United States,* [2]*Tufts University, Boston, MA, United States,* [3]*Massachusetts General Hospital, Boston, MA, United States,* [4]*International Center for Diarrheal Diseases Research, Bangladesh, Dhaka, Bangladesh*

Vibrio cholerae is a non-invasive gram-negative pathogen that causes cholera, resulting in over 100,000 deaths every year. In a recent study of the gut microbiota of infected individuals, a soil microbe *Paracoccus aminovorans* was found to be abundant during symptomatic cholera. In vitro, *V. cholerae* cultured in *P. aminovorans* spent culture supernatant (SCS) significantly increased the growth of V. cholerae. In this study, we characterized the effect of *P. aminovorans* on *V. cholerae* virulence factor expression. Compared to control microbiome species SCS, we found that V. cholerae grown in *P. aminovorans* SCS significantly increased expression of the toxin-coregulated pili and cholera toxin mRNA, measured by qPCR ($p<0.001$, nonparametric t-testing). We also observed increased biofilm formation by V. cholerae when co-cultured with *P. aminovorans* co-culture, and this effect was not present when *V. cholerae* was grown in *P. aminovorans* SCS alone ($p=0.03$). To investigate whether *P. aminovorans* could influence *V. cholerae* pathogenesis *in vivo*, we colonized mice with *P. aminovorans* and challenged them with *V. cholerae.* Colonization with *P. aminovorans* led to increased recovery of *V. cholerae* colony forming units in the small intestine ($p<0.005$). In summary, *P. aminovorans* directly enhances *V. cholerae* colonization within a host and could potentially exacerbate disease with increased expression of virulence factors. These results demonstrate that a species identified from the human gut microbiota during cholera could impact clinical *V. cholerae* infection.

9

SINGLE-CELL T CELL RECEPTOR ANALYSIS REVEALS CLONALITY OF MUCOSAL-ASSOCIATED INVARIANT T (MAIT) CELLS DURING *VIBRIO CHOLERAE* INFECTION

Taliman Afroz[1], Hasan Al Banna[2], Jahidul Islam[2], Md. Imran Hossain Bhuiyan[2], Ioana Pop[1], Owen Jensen[1], Ashraful I. Khan[2], Kaiissar Mannoor[3], Jason B. Harris[4], Stephen B. Calderwood[4], Edward T. Ryan[4], Firdausi Qadri[2], Taufiqur R. Bhuiyan[2], **Daniel T. Leung**[1]

[1]*Division of Infectious Diseases, University of Utah, Salt Lake City, UT, United States,* [2]*icddr,b, Dhaka, Bangladesh,* [3]*Institute of Developing Science and Health Initiatives, Dhaka, Bangladesh,* [4]*Massachusetts General Hospital, Boston, MA, United States*

Cholera is a mucosal infection with the potential to cause life-threatening acute watery diarrhea. MAIT cells are innate lymphocytes enriched at the mucosa, with a semi-invariant TCR V-alpha chain (Va7.2 or TRAV1-2). The activity and function of innate lymphocytes such as MAIT cells are not well-known during mucosal infections such as cholera. Previous work has shown a higher activation of MAIT cells at day 7 of cholera compared to day 2. Our objective was to determine the clonality of MAIT cells during *V. cholerae* O1 infection. We enrolled culture-confirmed cholera patients and obtained peripheral blood at days 2, 7, and 90. We used flow cytometry to sort MAIT cells, defined as CD3+ Va7.2+ CD161+ cells, followed by high-throughput bar-coded single-cell T cell receptor sequencing. In a limited sample of patients, we found that the most common TCR-alpha clonotypes were TRAJ33 and TRAJ34, and TCR-beta clonotypes were TRBV7-3 TRBJ2-2 and TRBV30 TRBJ2-2. In the peripheral blood, TCR-beta diversity, as assessed by Simpson diversity index, was higher at day 2 (n=8) than day 7 (n=5) and day 90 (n=2), with analysis of more patients pending. In addition, in a single patient with paired blood and duodenal biopsy samples obtained on the same day, we found that intestinal MAIT cells have a different clonal distribution than blood MAIT cells. These data suggest that blood MAIT cells may undergo clonal expansion during cholera infection, though these may not reflect the distribution of clonotypes in the intestinal mucosa. Additional data with a larger sample size will be available at the time of presentation.

11

INVESTIGATING CHOLERA TRANSMISSION DYNAMICS USING WHOLE GENOME SEQUENCING OF WATER AND CLINICAL *VIBRIO CHOLERAE* ISOLATES IN DHAKA, BANGLADESH (CHOBI7 TRIAL)

Christine Marie George[1], Matthew Dorman[2], K.M. Saif-Ur-Rahman[3], Shirajum Monira[3], Shirajum Monira[3], Md. Sazzadul Islam Bhuyian[3], Khaled Hasan[1], Fatema-Tuz Johura[3], Toslim T. Mahmud[3], Shan Li[4], Jessica Brubaker[1], Jamie Perin[1], Zillur Rahman[3], Munshi Mustafiz[3], David A. Sack[1], Munirul Alam[3], O Colin Stine[4], Nicholas Thomson[5], Daryl Domman[6]

[1]*Johns Hopkins University, Baltimore, MD, United States,* [2]*Wellcome Sanger Institute, Hinxton, United Kingdom,* [3]*International Center for Diarrheal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh,* [4]*University of Maryland School of Medicine, Baltimore, MD, United States,* [5]*Wellcome Sanger Institute and London School of Hygiene & Tropical Medicine, Keppel St, London, England, Hinxton, United Kingdom,* [6]*University of New Mexico School of Medicine and Wellcome Sanger Institute, Hinxton, United Kingdom, Albuquerque, NM, United States*

Household contacts of cholera patients have a 100 times higher risk of developing a cholera infection compared to the general population. To compare the genetic relatedness of *Vibrio cholerae* isolates from infected household contacts and water sources within patient households, we performed whole-genome-sequencing (WGS) of 446 isolate genomes (87 water and 359 clinical) across three cholera outbreaks in Dhaka, Bangladesh. Two isolates were collected on average from each water and clinical sample. Samples were collected from a total of 97 cholera patient households. Four hundred thirty five pandemic *V. cholerae* El Tor (7PET) (80 water and 355 clinical genomes) and 11 non-7PET isolates (7 water and 4 clinical genomes) genomes were identified. All 7PET genomes were genotypically *V. cholerae* O1 Ogawa and harbored the *ctxB1* variant. One sample from a cholera patient had both 7PET and non-7PET isolates, and two asymptomatic household contacts had non-7PET isolates. The median pairwise difference in single nucleotide variant (SNV) counts across all 7PET genomes was 7 ± 40.34 (standard deviation) (minimum- maximum: 0-588). We identified 14 7PET genomes that had a higher number of SNVs compared to the reference genome above the 97th percentile (>160 SNVs), suggestive of a hyper-mutator phenotype. Excluding these putative hyper-mutators, the median pairwise differences in SNV counts for 7PET genomes was 7 ± 5.34 (standard deviation) (minimum-maximum: 0-49). For 7PET genomes from the same sample the median pairwise SNV difference was 0 ± 0.59 (0-9). The median pairwise SNV difference for 7PET genomes collected from the same household was 0 ± 9.86 (0-337), compared to 7 ± 38.74 (0-579) for different households. The median pairwise SNV difference was similar for 7PET clinical (7 ± 37.96 (0-579)) and water genomes (5 ± 48.80 (0-253)). The majority of 7PET clinical and water genomes across these three outbreaks were closely related. These findings are highly suggestive of water-to-person and person-to-person transmission and demonstrate the need for interventions that promote both hygiene and water treatment.

12

INTESTINAL AND SYSTEMIC INFLAMMATION INDUCED BY SYMPTOMATIC AND ASYMPTOMATIC ENTEROTOXIGENIC *E. COLI* INFECTION IN AN EXPERIMENTAL CHALLENGE MODEL IN HUMANS

Subhra Chakraborty[1], Jessica Brubaker[1], A Louis Bourgeois[2], David Sack[1]

[1]*Johns Hopkins University, Baltimore, MD, United States,* [2]*PATH, Washington, DC, United States*

Given the importance of ETEC as one of the leading causes of diarrhea and stunting among children in the developing world with ~ 1 billion cases of diarrhea annually, better understanding of the impact of ETEC infection beyond diarrhea is much needed. Therefore, in this study, in an experimental challenge model in humans we evaluated if ETEC infection induces intestinal and systemic inflammation and if the magnitude and kinetics of inflammation are dependent on the dose and clinical outcome. In a dose descending experimental challenge model, healthy adult volunteers were challenged with a dose of 10^8, 10^7, 10^6 or 10^5 CFU of ETEC strain H10407. Subjects were monitored for signs and symptoms of enteric illness and stool and blood samples were collected to detect the levels of myeloperoxidase (MPO) and eleven pro-inflammatory cytokines respectively before and subsequent days after challenge. Following challenge, the volunteers who had moderate to severe diarrhea (MSD), the MPO levels in the stool increased significantly from day before challenge with the highest geometric mean (GM) fold increase of 19.8fold ($p<0.0001$) and peaked at 4958.59 pg/gm of stool on day 3. Among the volunteers who had no diarrhea the MPO levels reached peak late, on day 5 with 3.25fold increase ($p=0.0129$). When analyzed by dose both the MSD and no diarrhea groups showed dose dependent increase of the MPO levels. Interestingly, there was no significant difference in the levels of MPO between the volunteers with MSD and asymptomatic, when infected with high dose of ETEC. Evaluation of systemic inflammation showed that the GM of IL-17 increased significantly from the baseline, and peaked on day 4 (4.26 fold increase $p =0.0043$). The IL-17 level among MSD was 3.78fold higher ($p=0.0002$) compared to no diarrhea on day 4. Our study establishes that, ETEC infection induces significant inflammation both systemic and, in the gut and the levels of increase is clinical outcome and dose dependent. This study also implicates that asymptomatic ETEC infection if caused by higher dose of ETEC could induce significant inflammation in gut with the levels similar to cause by ETEC MSD.

13

PROTECTION OF MICE AGAINST ETEC-INDUCED DIARRHEA AND WEIGHT LOSS BY IMMUNIZATION WITH BI-VALENT RECOMBINANT TY21A TYPHOID ETEC VACCINE

Tint T. Wai[1], David T. Bolick[2], Minglin Li[1], Lixin Gao[1], Sumana Chakravarty[3], Eric R. James[3], Weiping Zhang[4], David A. Sack[5], Richard L. Guerrant[2], Stephen L. Hoffman[3], **B. Kim Lee Sim**[1]

[1]*Protein Potential, Rockville, MD, United States,* [2]*University of Virginia, Charlottesville, VA, United States,* [3]*Sanaria, Rockville, MD, United States,* [4]*University of Illinois, Urbana, IL, United States,* [5]*Johns Hopkins University, Baltimore, MD, United States*

Escherichia coli (ETEC) strains are a major cause of illness in children in the developing world and the number one cause of travelers' diarrhea. Despite decades of efforts, there is no FDA-licensed vaccine against ETEC. We used the existing live, oral, attenuated *Salmonella* Typhi Ty21, a typhoid vaccine that has an extensive safety record, as the vector to develop an oral, room-temperature stable, bi-valent vaccine against ETEC diarrhea and typhoid fever. A multi-epitope fusion antigen (MEFA) representing 7 separate CFA ETEC adhesins and the two toxins can be fused as a single protein (designated here as MEFA+T) and induces cross-protective antibodies that block adherence of heterogeneous ETEC strains to human colon cancer cells *in vitro*, and neutralizes two toxins in all ETEC strains. We created a recombinant Ty21a expressing MEFA+T (Ty21a-ETEC). It is composed of Ty21a expressing both heat-labile (LT) and heat stable enterotoxin (STa) and 7 adhesins [CFA/I, CFA/II (CS1-CS3) and CFA/IV (CS4-CS6)] stably chromosomally integrated and expressed as a holotoxin structured CFA-toxoid fusion cassette (termed MEFA+T) antigen in Ty21a (our Ty21a-ETEC vaccine). Intraperitoneal immunization of mice with live Ty21a-ETEC vaccine induced antibodies against LTB and MEFA that blocked binding to GM1 ganglioside (anti-toxin activity) and blocked adhesion of ETEC to intestinal epithelial Caco-2 cells (anti-adhesin activity) respectively. We tested our Ty21a-ETEC vaccine in a newly developed robust model in young mice that exhibit diarrhea and weight loss after challenge with ETEC. Mice immunized with Ty21a-ETEC significantly decreased, 1) numbers with diarrhea (62.5% protection, p equals 0.009), 2) diarrhea scores and 3) weight loss as compared to controls. We have also shown that foam-dried Ty21a-based vaccines are stable at ambient temperature for up to 20 months. This vaccine, which will be orally administered, has the potential to provide enormous public health benefits for residents of developing countries, protect travelers, and serve national biodefense programs against ETEC-mediated biowarfare and bioterrorism threats.

14

GUT MICROBIOME COMMUNITY COMPOSITION INFLUENCES ON DIARRHEA SYMPTOMS ASSOCIATED WITH *E. COLI* INFECTIONS

Kelsey J. Jesser[1], Angela Peña-Gonzalez[2], Janet K. Hatt[3], William Cevallos[4], Gabriel Trueba[5], Kostantinos T. Konstantinidis[3], Karen Levy[1]

[1]*Emory University, Atlanta, GA, United States,* [2]*University of Los Andes, Bogota, Colombia,* [3]*Georgia Institute of Technology, Atlanta, GA, United States,* [4]*Universidad Central del Ecuador, Quito, Ecuador,* [5]*Universidad San Franscisco de Quito, Quito, Ecuador*

A high proportion of *E. coli* infections in high transmission low- and middle-income country settings are asymptomatic for diarrhea, and characteristics of the gut microbiome may help to explain why. We conducted an analysis of 16S rRNA gene (16S) amplicon sequencing data for symptomatic and asymptomatic *E. coli* infections identified in whole stool samples collected during EcoZUR, an age-matched case-control study of diarrhea and the gut microbiome conducted in northern Ecuador. 16S data were collected for 202 symptomatic stool samples (diarrhea cases) and 207 asymptomatic samples (controls) in which an *E. coli* pathotype was isolated and PCR-typed. QIIME-2 was used to process 16S data and test for differences in alpha (within-sample) and beta (between-sample) diversity. LEfSe was used to identify taxa significantly associated with either infection type. Individuals with diarrhea had lower alpha diversity than those with asymptomatic infections across all *E. coli* pathotypes. There were no significant differences in beta diversity. We identified 43 taxa associated with symptomatic *E. coli* cases and 69 associated with asymptomatic infections. Specifically, members of the genus *Bacteroides* were enriched in symptomatic infections and members of the phylum *Proteobacteria* were more abundant in asymptomatic infections. *E. coli* pathotype-specific analyses revealed that asymptomatic ETEC infections were associated with increased relative abundances of taxa within the *Verrucomicrobia*. Asymptomatic atypical EPEC infections were linked to an increase in *Bacteroidetes*, and members of the *Enterobacteriaceae* were associated with symptomatic atypitcal EPEC infections. These data suggest that microbiome diversity in individuals may reflect differences between symptomatic and asymptomatic enteric infections both within and across *E. coli* pathotypes, and that there are specific taxa associated with symptomatic versus asymptomatic disease. We will also present results of current efforts to characterize the functional potential and *E. coli* population diversity and abundance in these samples using shotgun metagenome and qPCR data

16

RECOMBINANT PARAPROBIOTICS: A NEW PARADIGM FOR TREATING GASTROINTESTINAL NEMATODES OF HUMANS

Raffi Van Aroian[1], Hanchen Li[1], Ambily Abraham[1], David Gazzola[1], Yan Hu[2], Kelly Flanagan[1], Ernesto Soto-Villatoro[1], Florentia Rus[1], Zeynep Mirza[1], Gary Ostroff[1]

[1]*UMASS Medical School, Worcester, MA, United States,* [2]*Worcester State University, Worcester, MA, United States*

Gastrointestinal nematodes (GINs) of humans, *e.g.*, hookworms, whipworms, and *Ascaris*, negatively impact childhood growth, cognition, nutrition, educational attainment, income, productivity, and pregnancy. Hundreds of millions of people are currently targeted with mass drug administration (MDA) of donated benzimidazole (BZ) anthelmintics. However, BZ efficacy is suboptimal and reduced/low efficacy of BZs against all human GINs has been seen. The challenge with any anthelmintic for human MDA is daunting: it must be safe, effective, very inexpensive, stable without a cold chain, and massively scalable. *Bacillus thuringiensis* (Bt) crystal protein 5B (Cry5B) has anthelmintic properties that could potentially fill this void. The aim of this study is to achieve an API (Active Pharmaceutical Ingredient) form of Bt Cry5B compatible with the challenges of MDA listed above. We expressed Cry5B in asporagenous Bt during vegetative phase, forming cytosolic crystals. These **Ba**cteria with **C**ytosolic **C**rystals (BaCC) were rendered inviable (inactivated BaCC or IBaCC) with food-grade essential oils. IBaCC potency and uptake was demonstrated and validated *in vitro* against nematodes. IBaCC was also potent *in vivo* against experimental human hookworm infections in hamsters. IBaCC was successfully scaled up to 350 liter production at a contract manufacturing facility with full bioactivity. Bioactivity of a simple fit-for-purpose enteric formulation and powdered IBaCC were successfully demonstrated in hamsters and mice against two different GINs. A small-scale histopathology study showed that five daily doses of 200 mg/kg Cry5B IBaCC (the curative single dose is 40 mg/kg) was completely safe. IBaCC is a safe, inexpensive, highly effective, easy-to-manufacture, and scalable anthelmintic compatible with MDA that represents a new paradigm for treating human GINs.

17

MOLECULAR MODELING TO ANALYZE DIFFERENCES IN ALBENDAZOLE BINDING SITES OF THE BETA TUBULIN OF HUMAN SOIL TRANSMITTED HELMINTHS

Brian Medernach[1], Yash Gupta[2], Steven Goicoechea[2], Ravi Durvasula[2], **Prakasha Kempaiah**[2]

[1]*Loyola University Medical Center, Chicago, IL, United States,* [2]*Loyola University Chicago Stritch School of Medicine, Maywood, IL, United States*

Soil transmitted intestinal helminths (STH), belonging to phylum Nematoda (roundworms), infect ~1.5 billion people worldwide annually. These include *Ascaris lumbricoides, Trichuris trichiura* and hookworms *Necator americanus* and *Ancylostoma duodenale*. The morbidity from STH is related to infection intensity and can cause many issues including dietary deficiencies like iron deficiency anemia, delayed cognitive and physical development. STH are most common in places without modern sanitation and infection occurs through contaminated soil, food and water with eggs. Albendazole is one of the primary benzimidazoles used as an anti-helminthic agent across the globe. Research has shown there is variability in the effect of albendazole on the egg reduction and cure rate of Albendazole on the different human STH. The literature has shown lower efficacy of Albendazole against *T. trichiura* and the hookworms compared to *A. lumbricoides*. Studies have shown that single nucleotide polymorphisms (SNPs) within helminths β-tubulin (Target) coding regions are associated with Albendazole resistance. The aim is to provide evidence of variability in the β- tubulin binding site pocket of the STH that may explain the variable efficacy of Albendazole on these parasitic helminths. The NCBI database provided known genomic sequences of the β-tubulin molecules for the human STH: *A. lumbricoides, T. trichiura, A. duodenale* and *N. americanus*. These gene sequences were than submitted for protein structure and function prediction utilizing I-TASSER (Iterative Threading ASSEmbly Refinement). Once models were obtained, the Albendazole-beta tubulin binding was simulated utilizing Schrödinger chemical simulation software. This modeling of the albendazole and beta tubulin interaction has given insight into differences that would allow for modification of albendazole to overcome resistance and improve efficacy against *T. trichiura* and the hookworms *A. duodenale* and *N. americanus*.

18

EFFICACY AND SAFETY OF ALBENDAZOLE AND HIGH-DOSE IVERMECTIN TREATMENT COMBINATION IN CHILDREN WITH *TRICHURIS TRICHIURA* INFECTION

Gabriela Matamoros[1], Ana Sanchez[2], Samary Rodriguez[3], Andres Escalada[4], Ramiro Aveldaño[4], María Mercedes Rueda[3], Carol Rodriguez[3], Maritza Canales[3], Marisa Juarez[4], Pamela Cajal[4], Alejandro Krolewiecki[4]

[1]*Instituto de Investigaciones en Microbiologia, Universidad Nacional Autonoma de Honduras, Tegucigalpa, Honduras,* [2]*Brock University, St. Catharines, ON, Canada,* [3]*Universidad Nacional Autonoma de Honduras, Tegucigalpa, Honduras,* [4]*Instituto de Investigaciones de Enfermedades Tropicales, Universidad Nacional de Salta, Salta, Argentina*

Trichuris trichiura is a gastrointestinal soil transmitted helminth infecting approximately 450 million people worldwide. Preventive chemotherapy with single-dose benzimidazoles has been the main recommended control strategy in endemic regions. Benzimidazoles have demonstrated low efficacy against this parasite, hence combination treatment has been proposed. The aim of this study was to assess the efficacy and safety of administering high dose of ivermectin (IVM) combined with albendazole (ABZ) to children. A Phase II non-inferiority randomized clinical trial was done in a rural, STH endemic community in northern Honduras. Children infected with *T. trichiura*, were enrolled and randomly assigned to receive one of the four following treatments: (1) single-dose ABZ 400 mg; (2) single-dose ABZ 400 mg + IVM 600µg/kg; (3) ABZ 400 mg for 3 consecutive days; or (4) ABZ 400 mg +IVM 600µg/kg for 3 consecutive days. The efficacy outcome was measured through egg reduction rate (ERR) and Cure Rate (CR), both assessed from baseline to 14-21 days after treatment using the Kato-Katz method. Safety was evaluated by analyzing the frequency and severity of adverse effects (AE) and severe adverse effects (SAE). This study was approved by Honduran and Canadian research ethics boards and is registered at CT.gov (NCT04041453). A total of 124 children were enrolled in the study, 123 completed the treatment and 117 follow-up (Group 1: 24, Group 2: 35 Group 3: 18 and Group 4: 40). The ERR for single-dose ABZ 400 mg, single-dose ABZ 400 mg + IVM 600µg/kg, ABZ 400 mg for 3 d, and ABZ 400 mg + IVM 600µg/kg for 3 d were: 50.41%, 96.72%, 72.12% and 100%, respectively; with p-values <0.001 between IVM containing groups and ABZ only arms. The CR for the treatment groups were 4%, 89%, 33% and 100%, respectively. No SAE were reported, 12 (9.6%) participants reported at least 1 AE and 6 (4.8%) AE were classified as related to treatment administration. The results of this study demonstrate the superior efficacy of ivermectin/albendazole combination treatments, as well as the safety of high dose ivermectin in children under 15 years of age and a worrisome low efficacy of ABZ monotherapy.

19

COMPARISON OF RESULTS FROM SCHOOL AND COMMUNITY-BASED SURVEYS ASSESSING THE IMPACT OF PREVENTIVE CHEMOTHERAPY FOR SOIL-TRANSMITTED HELMINTHIASIS CONTROL

Sanjaya Dhakal[1], Md. Jahirul Karim[2], Abdullah Kawsar[2], Cara Tupps[3], Rubina Imtiaz[3]

[1]*The Task Force for Global Health, Decatur, GA, United States,* [2]*Elimination of Lymphatic Filariasis & STH Control Program, Dhaka, Bangladesh,* [3]*Children Without Worms, Decatur, GA, United States*

For Soil-Transmitted Helminth (STH) control, endemic countries follow World Health Organization's (WHO) guidance to target school age children (SAC) with preventive chemotherapy (PCT) and subsequent impact assessment surveys are also conducted usually by sampling SAC. Recently community-level impact assessment surveys were conducted by Children Without Worms (CWW) with the Ministry of Health and Family Welfare's Program on Lymphatic Filariasis Elimination and STH (ELFSTH) to estimate impact across all ages in endemic, treated communities. We present a comparison of prevalence results among SAC across both methods. School-based surveys (SBS) were powered to the national level and systematically sampled 50 children aged 5-12 years, from 1 or more schools per district. The community-wide surveys (CWS) powered to the district level, were implemented in 10 districts using multi-stage cluster sampling and population proportional to estimated size, and assessed STH prevalence and intensity for all ages and parasites. Both surveys used Kato Katz was stool lab analyses. We compared STH prevalence and intensity for children aged 5-12 years in both surveys. The SBS tested 5,300 students from 106 schools in 63 districts. The community survey tested 10,824 individuals from 10 districts, of which 3,196 were 5-12 years old. The STH prevalence was substantially higher in the CWS compared to SBS (13% vs. 8.1%) for the 10 compared districts with both surveys. The prevalence of moderate-to-high intensity infection was also higher in CWS compared to SBS (3.7 vs. 1.73%).The community-wide survey appears to be more generalizable in measuring STH prevalence and intensity in school aged children, as it is powered to the level of PCT implementation. School-based surveys are probably useful for monitoring in earlier phases of PCT programs, but later, there is a need for greater rigor in sampling to yield granular, reproducible and valid data. Population-representative surveys can produce high quality data for decisions around changing PCT frequency and/or target populations.

20

ACANR3990, GENOME MINING LEADS TO AN IMPROVED RAT LUNGWORM PCR

William Sears[1], Yvonne Qvarnstrom[2], Eric Dahlstrom[3], Jan Slapeta[4], David Modry[5], Vojto Balaz[5], Lisa Kaluna[6], Kirsten Snook[7], Susan Jarvi[7], Thomas B. Nutman[1]

[1]*NIAID, Bethesda, MD, United States,* [2]*CDC, Atlanta, GA, United States,* [3]*RML Genomics, Hamilton, MT, United States,* [4]*The University of Sydney, Sydney, Australia,* [5]*University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic,* [6]*Daniel K. Inouye College of Pharmacy, University of Hawai'i at Hilo, Hilo, HI, United States,* [7]*Daniel K. Inouye College of Pharmacy, University of Hawai'i at Hilo, Hilo, HI, United States*

Angiostrongylus cantonensis (Ac), or the rat lungworm, is one of the major causes of eosinophilic meningitis in humans. Humans get infected by ingesting food contaminated with the infective 3rd stage larvae that develops in many species of snails and slugs. Typically, the diagnosis of angiostrongyliasis is made based on clinical symptoms and evidence of eosinophilia in the cerebrospinal fluid (CSF). CDC and the Hawaii Public Health Laboratory offer PCR-based testing on CSF, using a qPCR assay targeting the ITS1 region, but its sensitivity is only ~70-80%. With the goal of developing a more sensitive assay, the genome of *A. cantonensis* was mined and primers and probes were designed to target the most repeated regions across the Ac genome. Using dilutions of genomic DNA (gDNA) of Ac, the analytical sensitivity of the best primer/probe combination, AcanR3990, was 1 fg (the DNA equivalent of 1/100,000 dilution of a single larvae). Moreover, this qPCR assay failed to amplify genomic DNA from all related Angiostrongylus species (except Angiostrongylus mackerrasae) and from less related nematode species. This assay was then tested using 128 archived CSF samples from patients with eosinophilic meningitis. The AcanR3990-based assay detected the presence of *Ac* in 56/56 CSF samples from confirmed angiostrongyliasis patients along with 3 samples where ITS1-based qPCR was negative despite very strong clinical and epidemiologic indications of angiostrongyliasis. . These cases were from varied geographic locations across North American and Asia. CSF from patients with neurocysticercosis, toxocariasis, gnathostomiasis and baylisascariasis each tested negative in this AcanR3990-based assay. In addition, infected Intermediate hosts (gastropods), paratenic hosts (gecko, mongoose), and an accidental animal host (a horse) were also tested and shown to detect Ac with great sensitivity across all sample sources. In conclusion, these results suggest that this new AcanR3990-based RtPCR assay is highly sensitive and specific and has the potential to be a widely applicable One Health detection method for Ac.

22

PREVALENCE, SEASONAL TREND, AND CLINICAL SEVERITY OF *CRYPTOSPORIDIUM*-ASSOCIATED DIARRHEAL DISEASE IN UNDER FIVE CHILDREN IN THREE SUB-SAHARAN AFRICAN COUNTRIES: RESULTS FROM THE VACCINE IMPACT ON DIARRHEA IN AFRICA (VIDA) STUDY, 2015-2018

M. Jahangir Hossain[1], Helen Powell[2], Leslie P. Jamka[2], Samba Sow[3], Richard Omore[4], Jennifer Verani[5], Joquina Chiquita M. Jones[1], Syed M.A. Zaman[1], Henry Badji[1], Stephen R. C. Howie[1], Golam Sarwar[1], Irene Kasumba[2], Uma Onwuchekwa[3], Sanogo Doh[4], Alex Ondeng[4], Sharon M. Tennant[2], Dilruba Nasrin[2], Anna Roose[2], Jie Liu[6], James Platts-Mills[6], Martin Antonio[1], Eric Houpt[6], Karen L. Kotloff[2]

[1]*Medical Research Council Unit The Gambia at the London School of Hygiene & Tropical Medicine, Banjul, Gambia,* [2]*Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States,* [3]*Center for Vaccine Development-Mali, Bamako, Mali,* [4]*Kenya Medical Research Institute, Center for Global Health Research (KEMRI-CGHR), Kisumu, Kenya,* [5]*Division of Global Health Protection, US Centers for Disease Control and Prevention, Nairobi, Kenya,* [6]*Division of Infectious Diseases and International Health, Department of Medicine, University of Virginia, Charlottesville, VA, United States*

Cryptosporidium, a protozoan parasite, is a leading cause of diarrhea and death in children globally. We describe prevalence, severity, and seasonality of *Cryptosporidium* in children <5 after rotavirus (RV) vaccine introduction in The Gambia, Mali, and Kenya. The Vaccine Impact on Diarrhea in Africa (VIDA) is a matched case-control study of children 0-59 months. Moderate-to-severe diarrhea (MSD) cases, defined as ≥3 loose stools/24 hours and ≥1 of: sunken eyes, poor skin turgor, dysentery, intravenous rehydration, or hospitalization, within 7 days of onset. Controls were diarrhea free for the preceding 7 days. Stool samples were collected and tested for enteropathogens by quantitative polymerase chain reaction (qPCR), including 18s rRNA for *Cryptosporidium* species, with qPCR cycle cut-off values <35 considered positive. Using episode-specific adjusted attributable fractions (AFes) we determined the etiology for each MSD case using a cut-off of AFe ≥0.5. We compared the severity of etiologic *Cryptosporidium* with RV cases and other causes of watery diarrhea using a 20-point modified Vesikari score (MVS). Among 4765 cases and 4775 controls, 1106 (23.2%) and 873 (18.3%) were positive for *Cryptosporidium*, respectively. *C. hominis* was the predominant species and higher in cases than controls (37.6% vs. 19.9%). *Cryptosporidium* was most often detected in case children 6-23 months (77.1%) and peaked annually in The Gambia and Mali during the rainy season. The median *Cryptosporidium* MVS was lower than RV (10 vs. 11, p <0.001), but the same as other causes of watery diarrhea (10 vs. 10, P <0.071). Of *Cryptosporidium* and RV-attributed cases, 87.8% and 90.7% were clinically moderate-to-severe episodes as defined by MVS. *Cryptosporidium* was associated with prolonged (≥6 days) diarrhea compared to RV (p

<0.001) and other causes of watery diarrhea (p <0.001). *Cryptosporidium* is an important pathogen causing MSD and prolonged watery diarrhea in sub-Saharan Africa. *Cryptosporidium* has similar clinical severity with other causes of watery diarrhea and affects younger ages. These data are important to prioritize development of new drugs and vaccines.

23

EPIDEMIOLOGY OF INFECTION WITH *BLASTOCYSTIS HOMINIS* AND ASSOCIATED OUTCOMES IN SLUM-DWELLING MALNOURISHED ADULTS IN BANGLADESH

Shah Mohammad Fahim[1], Md. Amran Gazi[1], Md. Ashraful Alam[1], Subhasish Das[1], Mustafa Mahfuz[1], M Masudur Rahman[2], Rashidul Haque[1], Shafiqul Alam Sarker[1], Tahmeed Ahmed[1]

[1]*icddr,b, Dhaka, Bangladesh,* [2]*Sheikh Russel National Gastro Liver Institute and Hospital, Dhaka, Bangladesh*

Blastocystis hominis (*B. hominis*) is a widely distributed gastrointestinal protozoan commonly reported in the countries with tropical and sub-tropical climate. However, the epidemiology, pathogenicity and associated outcomes of the organism remain ambiguous. The aim of this analysis was to determine the factors associated with *B. hominis* infection in malnourished adults living in a slum in Dhaka, Bangladesh. We also sought to investigate the role of *B. hominis* on the biomarkers of intestinal health among the study participants. Total 316 malnourished adults with a body mass index ≤18.5 kg/m^2 were included in the analysis. The presence of *B. hominis* in feces was evaluated using TaqMan Array Cards. *B. hominis* was detected in 80.4% of the enrolled participants. The prevalence was significantly higher in female than male (83.5% vs. 72.1%, p=0.03), in younger adults aged ≤20 years (84.4%, p=0.04), and in those living in households with high crowding index (94.2%, p=0.01). The prevalence of infection with *Helicobacter pylori* (82% vs. 68%, p=0.03), *Shigella* (51% vs. 36%, p=0.025), EAEC (64% vs. 45%, p=0.007), and *Trichuris trichiura* (28% vs. 16%, p=0.047) were estimated significantly greater in adults with *B. hominis* infection than those with no infection. Malnourished adults who were anemic (aOR = 4.48; 95% CI = 1.55, 16.49; p=0.01) and infected with *Helicobacter pylori* (aOR = 2.72; 95% CI = 1.10, 6.77; p=0.03) were more likely to be infected with *B. hominis*. Living in households with low (aOR = 0.04; 95% CI = 0.002, 0.35; p=0.01) and moderate (aOR = 0.10; 95% CI = 0.01, 0.51; p=0.03) crowding index had lower odds of being infected by *B. hominis*. Multivariable linear regression analyses showed that *B. hominis* had significant negative relationship with neopterin (β = -12768.4; 95% CI = -24488.6, -1048.1; p=0.03), alpha-1 antitrypsin (β = -0.2; 95% CI = -0.2, -0.1; p<0.001) and Reg1B (β = -7.5; 95% CI = -12.2, -2.8; p=0.002) concentrations measured in stool samples of the malnourished adults. The study findings suggest that the presence of *B. hominis* in human intestine influences gut health and may have potential pathogenic role in presence of other pathogens.

25

NANOPARTICLE-ASSISTED DETECTION OF OPPORTUNISTIC *TOXOPLASMA GONDII* INFECTIONS IN PLHIV THROUGH NOVEL MASS SPECTROMETRY BASED ANTIGEN DISCOVERY

Hannah E. Steinberg[1], Andrea Diestra[2], Cusi Ferradas[2], Maritza Calderon[2], Catherine Apaza[2], Marilly Donayre Urquizo[3], Daniela E. Kirwan[4], Lilia Cabrera[5], Freddy Tinajeros[6], Viviana Pinedo Cancino[7], Lastenia Ruiz[3], Cesar Ramal[8], Paul Russo[9], Nancy Freitag[1], Natalie M. Bowman[10], Lance A. Bowman[9], Alessandra Luchini[9], Robert H. Gilman[6]

[1]*University of Illinois, Chicago, Chicago, IL, United States,* [2]*Universidad Peruana Cayetano Heredia, Lima, Peru,* [3]*Universidad Nacional de la Amazonía Peruana, Iquitos, Peru,* [4]*St George's, University of London, London, United Kingdom,* [5]*AB Prisma, Lima, Peru,* [6]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [7]*Universidad Nacional de la Amazonía of Illinois, Chicago, Iquitos, Peru,* [8]*Hospital Regional de Loreto, Iquitos, Peru,* [9]*George Mason University, Fairfax, VA, United States,* [10]*University of North Carolina, Chapel Hill, NC, United States*

Opportunistic neurological infections in persons with HIV (PLHIV) are challenging to diagnose. CT and MRIs with a clinical suspicion can guide treatment, but definitive diagnosis remains elusive for many patients. *Toxoplasma gondii*is known to cause Toxoplasmic Encephalitis in PLHIV. Though difficult to diagnosis, it has up to 90% clinical response rate to treatment. Our team has developed a diagnostic western blot with a hydrogel nanoparticle concentration step in urine for*T. gondii* antigen. The nanoparticle concentration step allows for the detection of as little as 7.8pg/ml of GRA1- a secretory antigen and 31.1pg/ml of SAG1- a surface antigen. GRA1 appears to be present in the urine of 50% of CSF qPCR positive patients, thus we needed further studies to identify more antigens to detect active *T. gondii* infection. Our team then has used a combination of hydrogel nanoparticle antigen concentration and discovery mass spectrometry to identify 334 peptides from urine and CSF specimens of PLHIV and clinical findings consistent with toxoplasmic encephalitis. Network analysis of these peptides revels 13 peptides with high degree centrality, suggestive of their potential as diagnostic targets. Further investigation of these peptides may yield novel antigen for detection of active TE infection.

28

OLD PARASITE, NEW LESSONS: HOW SECULAR CHANGES IN *TOXOPLASMA GONDII* ENDEMICITY INFLUENCE THE INCIDENCE OF CONGENITAL DISEASE IN HUMAN POPULATIONS

Gregory Milne, Joanne P. Webster, Martin Walker

Royal Veterinary College, Brookmans Park, United Kingdom

The apicomplexan protozoan parasite *Toxoplasma gondii*, the aetiological agent of toxoplasmosis, is among the most ubiquitous parasites worldwide, capable of infecting humans and all warm-blooded animals. *T. gondii* is transmitted horizontally, either by consumption of cyst-stage bradyzoites in undercooked/raw meat, or by ingestion of environmental-stage oocysts shed in the faeces of infected felids. It is also transmitted vertically from mother to child, usually when a woman is newly exposed (seroconverts) to *T. gondii* during pregnancy. Congenital transmission causes abortions and a range of life-long ocular and craniocerebral sequelae that currently account for the bulk of the disease burden associated with toxoplasmosis. Although infection with *T. gondii* has been declining in a number of countries around the world, an epidemiological consequence of reduced transmission is that more women may become first exposed to the parasite at peak childbearing ages. This may result in more seroconversions occurring during pregnancy, potentially increasing the incidence of abortion and congenital disease. Here we developed an age-structured mathematical model to investigate how temporal reductions in the force of infection of *T. gondii* affect the incidence of congenital transmission and disease. We fitted the model to temporally resolved age-structured country-specific seroprevalence data to estimate changes in the force of infection and we made projections on the incidence of congenital disease using demographic and fertility data. We demonstrate how secular declines in the force of infection can lead to increases in the incidence of congenital disease, depending on the particular demographic and epidemiological context. We discuss our results in the context of screening programmes for pregnant women and the potentially underestimated disease burden of this globally pervasive parasite.

29

SAFETY AND IMMUNOGENICITY OF ACCELERATED HETEROLOGOUS TWO-DOSE EBOLA VACCINE REGIMENS IN HIV-INFECTED AND HIV-UNINFECTED ADULTS IN AFRICA

Betty Mwesigwa[1], Fredrick Sawe[2], Janet Oyieko[3], Nyanda Ntinginya[4], Joel Mwakisisile[4], Josphat Kosgei[5], Elizabeth Ngetich[6], Ilesh Jani[7], Edna Viegas[7], Kokogho Afoke[8], Akindiran Akintunde[9], Georgi Shukarev[10], Leigh Anne Eller[11], Michael Eller[12], Lucy Ward[13], Janice Rusnak[14], Callie Bounds[13], Christopher Badorrek[14], Christina Polyak[11], Lalaine Anova[12], Amber Moodley[11], Chi Tran[11], Melissa Van Alst[10], Dickson Anumendem Nkafu[15], Auguste Gaddah[15], Viki Bockstal[10], Kerstin Luhn[10], Macaya Douoguih[10], Cynthia Robinson[10], Prossy Naluyima[1], Monica Millard[16], Hannah Kibuuka[1], Julie Ake[17]

[1]*Makerere University Walter Reed Project, Kampala, Uganda,* [2]*U.S. Military HIV Research Program, Walter Reed Army Institute of Research/Henry M. Jackson Foundation Medical Research International, Kericho, Kenya,* [3]*Henry M. Jackson Foundation Medical Research International/Kenya Medical Research Institute/U.S. Army Medical Research Directorate-Africa/ Kenya, Kisumu, Kenya,* [4]*National Institute for Medical Research-Mbeya Medical Research Center, Mbeya, United Republic of Tanzania,* [5]*Kenya Medical Research Institute/U.S. Army Medical Research Directorate-Africa, Kericho, Kenya,* [6]*Kenya Medical Research Institute/U.S. Army Medical Research Directorate-Africa/Kenya, Kericho, Kenya,* [7]*Polana Caniço Health Research and Training Center, Maputo, Mozambique,* [8]*U.S. Military HIV Research Program, Walter Reed Army Institute of Research/Henry Jackson Foundation MRI/US Army Medical Research Directorate-Africa, Abuja, Nigeria,* [9]*U.S. Military HIV Research Program, Walter Reed Army Institute of Research/Henry Jackson Foundation MRI/US Army Medical Research Directorate, Abuja, Nigeria,* [10]*Janssen Vaccines and Prevention, Leiden, Netherlands,* [11]*U.S. Military HIV Research Program, Walter Reed Army Institute of Research/Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD, United States,* [12]*U.S. Military HIV Research Program, Walter Reed Army Institute of Research/Henry M. Jackson Foundation for the Advancement of Military Medicine, Silver Spring, MD, United States,* [13]*Joint Project Manager Chemical, Biological, Radiological, and Nuclear (CBRN) Medical, Fort Detrick, MD, United States,* [14]*Contractor for Joint Project Manager Chemical, Biological, Radiological, and Nuclear (CBRN) Medical, Fort Detrick, MD, United States,* [15]*Janssen Research and Development, Beerse, Belgium,* [16]*U.S. Military Research Directorate Africa, Kampala, Uganda,* [17]*U.S. Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, MD, United States*

Ebola vaccine trials in Africa have evaluated Ad26.ZEBOV and MVA-BN-Filo 2 dose regimens. Strongest immune responses were induced with Ad26.ZEBOV followed by MVA-BN-Filo 2 months later. Shorter regimens are more conducive to outbreak settings. EBL2003 Part 2 was a Phase 2 study of accelerated 2 dose regimens of Ad26.ZEBOV and MVA-BN-Filo. It enrolled stable HIV infected and HIV uninfected adults in 5 African countries, evaluating Ad26.ZEBOV with MVA-BN-Filo given 28 days later (group 1) and MVA-BN-Filo with Ad26.ZEBOV given 14 days later (group 2). Primary objectives were safety and binding antibodies (abs) to the Zaire glycoprotein. 499 participants were randomized to groups 1 (n=400) or 2 (n=100) and to receive vaccine or placebo in a 4:1 ratio. Randomization was done separately for HIV infected and HIV uninfected adults. Vaccines were administered intramuscularly in 0.5 ml volumes of Ad26.ZEBOV at $5x10^{10}$ and MVA-BN-Filo at $1x10^{8}$. Solicited and unsolicited adverse events (AEs) were collected. Binding antibody responses were assessed via EBOV GP FANG ELISA. Study participants were 52.3% female, median age 32 years (range 18-67). Solicited AEs were generally mild or moderate. There were no vaccine related serious AEs and no remarkable safety profile differences between HIV infected and uninfected groups. At 21 days post dose 2, vaccine responders were 99% (group 1) and 97% (group 2) of the HIV uninfected participants, and 96% (group 1) and 100% (group 2) of the HIV infected participants. Binding abs persisted in 90% at 1 year. The geometric mean concentration (GMC) of EBOV-specific binding abs for group 1 HIV uninfected adults was 6037 EU/mL (95% CI 4996;7297) (n=151) and 2939 EU/mL (95% CI 2316;3729) (n= 155) for HIV infected adults. In group 2, HIV uninfected participants had GMC of 5168 (95% CI 2919; 9152) (n=32) and HIV infected participants had GMC of 2509 (95% CI 1701;3702) (n=37). The Ad26,MVA 28-day interval induced similar GMC as the MVA,Ad26 14-day interval. At 21 days post dose 2, there were lower responses in HIV infected versus uninfected adults - this difference did not persist at a year. Both regimens were safe and immunogenic in the African populations studied.

30

IMPLICATIONS OF ASYMPTOMATIC MALARIA INFECTIONS ON HEMATOLOGICAL PARAMETERS IN PEOPLE LIVING WITH HIV

Edwin Kamau[1], Risper Maisiba[2], Michael Iroezindu[1], Emmanuel Bahemana[1], Dennis Juma[2], Hannah Kibuuka[3], Nicole Dear[1], Allahna Esber[1], Ajay Parikh[1], Trevor A. Crowell[1], Julie A. Ake[1], John Owuoth[1], Jonah Maswai[1], Ben Andagalu[2], Benjamin Opot[2], Amanda L. Roth[2], Raphael O. Okoth[2], Farid Abdi[2], Marueen Mwalo[2], Christina S. Polyak[1]

[1]*US Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [2]*US Army Medical Research Directorate - Africa, Nairobi, Kenya,* [3]*Makerere University Walter Reed Project, Kampala, Uganda*

Acute febrile malaria is associated with hematologic abnormalities. However, asymptomatic malaria may also contribute to hematologic abnormalities, especially among people living with HIV (PLWH). We describe prevalence of asymptomatic malaria and hematological parameters in PLWH and in HIV-uninfected individuals in the African Cohort Study (AFRICOS). AFRICOS is an open-ended prospective cohort study enrolling PLWH and HIV-uninfected adults in PEPFAR-associated clinical sites in Kenya (Kisumu and Southern Rift Valley [SRV]), Uganda and Nigeria. Malaria parasitemia was diagnosed by PCR. Hematological parameters were analyzed using a Coulter Ac•T™ 5diff CP analyzer. Anemia was defined as Hb<11.0 g/dL. Logistic regression models with generalized estimating equations were used to estimate associations between clinical factors and parasitemia, stratified by HIV status.A total of 5734 samples collected between January 2013 and April 2020, including 4924 (85.9%) from PLWH and 809 (14.1%) from HIV-uninfected participants were analyzed. The odds of having parasitemia was significantly reduced (OR 0.58; p <0.001) in PLWH compared to HIV-uninfected, with parasitemia prevalence of 20.7% in PLWH compared to 31.0% in HIV-uninfected. In PLWH, ART status, ART class, duration on ART or viral load did not influence the prevalence of parasitemia. The odds of having parasitemia was lower in individuals taking cotrimoxazole (OR 0.7; p<0.001), and in PLWH, parasitemia prevalence differed based on CD4 category (p=0.04). Parasitemia was not associated with anemia but differed based on location (p=0.05). In PLWH, there was no difference in WBC, neutrophils, lymphocytes, eosinophil and basophil in aparasitemic compared to parasitemic individuals. However, the median MCV, platelet count and percent monocyte were significantly higher in PLWH who were aparasitemic compared to those who were parasitemic (p<0.007; p=0.02; and p=0.05 respectively). This study demonstrates asymptomatic malaria infections influence hematological parameters in PLWH, suggesting screening and treatment of asymptomatic malaria infections should be considered.

32

IMPACT OF ANTHELMINTIC THERAPY FOR INVASIVE HELMINTH INFECTION ON MICROBIAL TRANSLOCATION, INFLAMMATION, AND IMMUNE RESPONSE AMONG UGANDANS LIVING WITH HIV

Grace Turyasingura[1], Stefanie Sowinski[2], Miya Yunus[3], Rojelio Mejia[4], David Boulware[5], Bozena M. Morawski[5]

[1]*Indiana University, Indianapolis, IN, United States,* [2]*Gladstone Institutes, UCSF, San Francisco, CA, United States,* [3]*The AIDS Support Organization (TASO), Kampala, Uganda,* [4]*Baylor College of Medicine, Houston, TX, United States,* [5]*University of Minnesota, Minneapolis, MN, United States*

Microbial translocation is considered a major driver of chronic immune activation, which is responsible for HIV disease progression. Invasive parasitic gut nematodes also induce microbial translocation. We evaluated the impact of albendazole anthelmintic therapy on serum markers of microbial translocation and inflammation among concurrently helminth- and HIV-infected Ugandans. Participants were randomized to immediate or delayed 1200mg albendazole therapy, and followed for 1 month. Baseline stool analysis determined parasitic infection prevalence. Baseline and follow-up blood draws evaluated soluble CD14 (sCD14), C-reactive protein (CRP), and 10 pro-inflammatory cytokines. Parametric and non-parametric tests examined the change in biomarker concentrations over time and across randomization arms. We randomized 224 HIV-infected, antiretroviral therapy (ART)-experienced adults in Mbale, Uganda. 24 (10.7%) participants were infected with either *Necator americanus* or *Strongyloides stercoralis*, 12 in the immediate albendazole arm, and 12 in the delayed albendazole arm. We observed increased concentrations of CRP, interleukin (IL)-4, IL-6, IL-10, and tumor necrosis factor (TNF)-α among persons with current helminth infection relative to uninfected participants at baseline. Participants in the immediate therapy arm had higher sCD14 concentrations at follow-up relative to participants in the delayed arm. We did not observe effects of anthelmintic therapy on any other biomarker concentrations among helminth-infected participants. In conclusion, increases in sCD14 post-anthelmintic therapy in this cohort require further investigation in larger cohorts and for longer follow-up durations. However, incorporating anthelmintic therapy into regular adult HIV care may provide subtle health benefits in this potentially vulnerable population.

33

ASSESSING GAPS IN CARE FOR HIV-INFECTED PEOPLE LIVING WITH AIDS IN TWO HOSPITALS IN ETHIOPIA

Anteneh Zewde

University Of Minnesota, Minneapolis, MN, United States

AIDS is still common in sub-Saharan Africa, despite improving access to ART. Cryptococcosis and tuberculosis are the most common causes of death in HIV patients with CD4 count <200 cells/μL.From our 2015 data published in Clinical Infectious Disease Journal, we face 68% mortality from cryptococcal meningitis in Adama. Despite recommendations for rapidly initiating HIV treatment, many persons in sub-Saharan Africa present to care with advanced HIV disease. In 2017, the World Health Organization (WHO) recommended a package of care for persons with advanced HIV disease. The package consists of cotrimoxazole prophylaxis to prevent PCP pneumonia, Tuberculosis (TB) screening, and prophylaxis, Cryptococcal (CrAg) screening and treatment, rapid initiation of antiretroviral therapy (ART) (for naïve persons) and enhanced patient adherence counseling. These packages of care are based on expert recommendations and need to be validated in real-world health care settings. It is yet to be proven if the package of care will improve survival. Our project did a preliminary assessment in two hospitals in Ethiopia before the implementation of the WHO recommended package of care. We found 3926 persons were lost to follow up or dead out of total 13105 ever enrolled at Adama site/29.9%. From Asella site out of 9741 ever enrolled, 4230 were lost to follow up or dead, which is 43.4%. Of 1835 HIV-infected persons enrolled in HIV clinic between the two sites from 2015-2019, only 30 (<2%) patients had a CD4 count performed. Fifteen of those 30 (50%) had a CD4 cell count <200 cells/mcL, which is considered AIDS or advanced HIV disease -at high risk of death. This is compared to almost 100% CD4 testing at least once before 2015. The CD4 testing rate dropped after the September 2015 WHO recommendation for all HIV positive persons to start ART(Test and treat strategy). Due to this, our two sites lost funding for CD4 reagent. However, we all know CD4 count is still crucial for following up on patients, HIV staging, and initiation f prophylaxis and diagnosis of opportunistic infection. We have proposed to implement rapid CD4 testing to help implement the WHO package of care.

35

LESSONS LEARNED FROM COMMUNITY SENSITIZATION FOR HUMAN IMMUNODEFICIENT VIRUS TESTING AND FOLLOW-UP DURING PREGNANCY IN RURAL AREA OF SOUTHERN MOZAMBIQUE

Felizarda E. Nhacolo, Salesio Macuacua, Anifa Valá, Esperança Sevene, Khátia Munguambe

Manhica Health Research Center, Manhica, Mozambique

The burden of HIV in Mozambique remains high. Late start of antiretroviral treatment as well as late initiation of antenatal care (ANC) visits among pregnant women contribute to inconsistent adherence patterns leading to challenges in HIV control strategies. We present lessons learnt from a community sensitization on importance of early initiation of ANC visits, HIV testing and treatment in Southern Mozambique. The community sensitization activities were nested in a study aiming to examine the safety of antiretroviral drugs during pregnancy in Southern Mozambique. The sensitization aimed to raise community awareness on the importance of early and regular ANC visits, HIV testing, ART adherence and family members involvement in pregnancy. Data were collected from 924 meetings involving 11,245 participants: women of reproductive age (WRA), pregnant women, partners, mothers and mothers-in-law. Data were registered in forms, entered in REDCap database and analysed using R Software and content analysis in Excel matrix. The participants were aware early ANC debut, including routine HIV testing for pregnant woman to protect their babies. However, WRA and pregnant women revealed hesitancy in disclosing HIV status to partners due to fear of partners' aggressive reaction, including expelling from home and prohibiting to comply with ART treatment. Exposure at ART collecting points and linked discrimination drives them to collect their drugs in health facilities far from their communities to ensure their confidentiality, incurring travel and time-consuming costs. This jeopardizes both ANC attendance and ART adherence. Participants consider that elders in the family should help raise awareness among their sons and daughters to comply with hospital recommendations. The study highlights awareness of ANC and vertical transmission prevention, but the stigma and lack of family support, especially from male partners become important barriers to the adequate use of these services. Concrete actions focused on engaging partners and family members are suggested.

36

PREVALENCE OF HBV, HCV, HIV AND SYPHILIS INFECTIONS AMONG SECONDARY SCHOOL STUDENTS IN JUBA, SOUTH SUDAN

Kenneth L. Sube[1], Oromo Seriano[1], Joseph Lako[1], Charles Ochero[2], Anthony Lasuba[1], Emmanuel Lino[3], Philip Abongo[1], Akech Simon[1], Ekal Lolup[1], Richard Lino[3]

[1]*University of Juba, Juba, South Sudan,* [2]*Royal Infirmary of Edinburgh, Edinburgh, United Kingdom,* [3]*National Ministry of Health, Juba, South Sudan*

Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Human Immunodeficiency Virus (HIV infections share similar transmission routes through contaminated blood, blood products and needles, and sexual contacts. Co-infection with HBV and HCV leads to an acceleration of fibrosis leading

to a faster progression to end-stage liver disease. In a 2014 study of blood donors, the prevalence of HIV was 7.9%. Co-infections between HIV and HBV, HCV and syphilis were 50%, and 18% respectively. In 2016, a study also of blood donors found a prevalence of HBV at 8.2% and HCV at 5.6%. This cross-sectional study was conducted at Juba Day Secondary School in Juba, South Sudan, between July - October 2017 to determine the prevalence of HBV, HCV, HIV and syphilis infections among the students. Data were obtained using standardized questionnaires, and laboratory tests were done and analyzed using SPSS. A total of 158 participants were included in the study. There were 80 (50.6%) males and 78 (49.4%) females, with the mean age of 19 years (SD+/-2.49). The prevalence of HBV, HCV, HIV and syphilis was 28%, 3.3%, 0% and 13.3% respectively, with the age group of 21-25 years more affected but not statistically significantly (p>0.05). Males (75%) were more affected than females (25%) with HBV while HCV occurrence was the same in each gender group. In the school with four-class grades, senior four has HBV at (50%) and syphilis (63%) prevalence. By location, the highest prevalence of HBV and syphilis (45% and 62.5%) respectively were found among students who stayed at Kator Residential Block while HCV was equally distributed in Kator (50%) and Munuki (50%) Residential Blocks but not statistically significant (p>0.05). There was no case of co-infection in this study. This study has highlighted a serious public health issue among this group of sexually active secondary school students. Without effective interventions in sexual and reproductive health as a matter of urgency, there will be an additional burden on healthcare provision in the near future.

37

EXPERIENCES STRENGTHENING MICROBIOLOGICAL LABORATORY CAPACITY IN RURAL RWANDAN HOSPITALS TO CATALYZE ROBUST NATIONAL ANTIMICROBIAL STEWARDSHIP PROGRAMS

Grace Umutesi[1], Lotta Velin[2], Moses Muwanguzi[1], Gilbert Rukundo[1], Aniceth Rucogoza[3], Carol Mugabo[1], Kara Faktor[2], Christian Mazimpaka[1], Jean de Dieu Gatete[1], Marthe Yankurije[1], Bethany Hedt-Gauthier[4], Robert Riviello[5], Tharcisse Mpunga[6], Emil I. Mwikarago[3], Fredrick Kateera[1]

[1]*Partners in Health/ Inshuti Mu Buzima, Kigali, Rwanda,* [2]*Program in Global Surgery and Social Change, Harvard Medical School, Boston, MA, United States,* [3]*National Reference Laboratory, Rwanda Biomedical Center, Kigali, Rwanda,* [4]*Department of Global Health and Social Medicine, Harvard Medical School, Boston, MA, United States,* [5]*Center for Surgery and Public Health, Brigham and Women's Hospital, Boston, MA, United States,* [6]*Butaro District Hospital, Ministry of Health, Butaro, Rwanda*

Antimicrobial Resistance (AMR) is a global public health threat. Human resource and capacity in diagnostics of common microbes and their resistance profiles is crucial to inform rational antibiotic use and set up of an Anti-microbial stewardship (AMS) system. This being especially important in LMICs where there is a paucity of such data. We sought to assess the capacity and describe our experience setting up microbiological diagnostic capacity at three rural district hospitals (DHs) as steps to establishing an AMS program in Rwanda. Partners in Health/ Inshuti Mu Buzima in collaboration with the National Reference Laboratory (NRL) and three DHs- Rwinkwavu, Kirehe, and Butaro- conducted a cross-sectional study to characterize bacteria and AMR profiles among patients diagnosed with open wound infections. Firstly, a laboratory needs assessment was conducted, followed by the requisitioning of equipment and the training of two laboratory technicians on culture media preparation, culturing techniques and sample processing at each hospital. Thereon, trained study nurses enrolled patients from different wards, swabbed their wounds in alignment with standardized procedures and samples were cultured at the hospital's laboratory within 24 hours. Eighteen to twenty-four hours later, the cultures were examined for growth and cultures transported to the NRL in Kigali for further testing using a VITEK 2 system. Essential supplies were difficult to find locally. In particular, sheep's blood was procured from the US but found contaminated at delivery and we ended up using human blood from the national blood bank as an alternative option. Overall, we realized that integrated processes within the different arms of the health system were needed to provide timely and accurate microbiological testing. For 115 samples used to compare the analysis performed at DHs to the one done at the NRL, we found a 73.9% concordance in growth assessment. Despite challenges observed, this pilot study demonstrates that it is feasible to establish microbiological testing in rural Rwandan hospitals, a capacity needed for the establishment of robust national AMS programs.

38

HEALTH SEEKING BEHAVIOUR FOR BURULI ULCER DISEASE IN THE OBOM SUB-DISTRICT OF THE GA SOUTH MUNICIPALITY OF GHANA

Eric Koka, Hannah Benedicta Taylor Abdulai

University of Cape Coast, Cape Coast, Ghana

The current biomedical Buruli ulcer case management strategies emphasise the importance of early reporting and appropriate medical treatment of nodules before they ulcerate and give rise to deformities and disabilities. However, there are a wide range of factors that influence health seeking behaviour for Buruli ulcer case management. The purpose of the study was to determine health seeking behaviour for Buruli ulcer by affected persons and their families. This was a descriptive study involving both qualitative and quantitative data collection. Thirty (30) in-depth interviews were conducted for Buruli ulcer patients and their corresponding caregivers on barriers and facilitators to health seeking. Three (3) Focus Group Discussions (FGDs) were also conducted among elderly community members. Survey questionnaire interviews were conducted with 300 community respondents in Ga, Akan and Ewe languages in the study area. Systematic sampling was used to select 300 respondents for the survey. The study revealed that most respondents (41.0%) would resort to self-medication as their first treatment option when infected with Buruli ulcer. However, the health seeking of self-medication before seeking biomedical treatment was alarming since it leads to delays in reporting. This is a serious public health concern since delay in reporting could lead to category three lesions.

39

ANTIOXIDANT ACTIVITY OF FLAVONOIDS FROM THE LEAVES OF *TAPINANTHUS PENTAGONIA*, LORANTHACEAE

Hermia Nalova Ikome

Institute of Medicinal Plants and Traditional Medicine, Yaounde, Cameroon

This work represents an extensive phytochemical study on Mistletoe (Loranthaceae) which will contribute to the chemotaxonomy of this important group of plants. Loranthaceae are flowering, chlorophyll and hemi-parasitic epiphytes that are located on the aerial parts of their hosts and are responsible for economic damage, ecological, technological and morphogenetic varied crops or the woody species parasitized The results obtained support the fact that Loranthaceae though known to cause damage to host plants also play an important role in the traditional pharmacopoeia Chemical investigation of the crude methanolic extract of the leaves of *Tapinanthus pentagonia* (**TP**), a Loranthaceae, resulted in the isolation of four known flavonoids namely (3-methoxy 5,7,4′-trihydroxyflavone (**1**), Quercetin 4′-methylether (**2**), (Quercetin-3-*O*-rhamnoside (**3**) and 3-*O*-rhamnosyl-5,7-dihydroxyl-3′,4′-methylenedioxyflavone (**4**). The structures of the isolated compounds were elucidated on the basis of extensive spectroscopic analysis including IR, UV, 1D and 2D-NMR as well as HRMS data. Three different methods namely; DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, ABTS scavenging activity and ferric reducing antioxidant power (FRAP) were used to assess the antioxidant activity of the crude extract together with the three of the isolated compounds (**1**, **3** and **4**) using catechin, ascobic acid and gallic acid as standards respectively. Results of our findings revealed that compound **3** exhibited the highest antioxidant activity in the three test based on IC_{50}its values followed by compound **4**. For the radical scavenging activity, compound **3** was the most active with an IC_{50}

of 0.024 mg/ml comparable to the IC_{50} of catechin which is 0.015 mg/ml. In summary, compounds **1**, **3** and **4** exhibit good antioxidant properties and reducing power compared to the methanolic extract. This could be explained by the phenolic nature of the tested compounds.

40

KNOWLEDGE AND PRACTICES OF MEDICAL SHOP WORKERS IN NEPAL IN THE DIAGNOSIS AND TREATMENT OF CORNEAL INFECTIONS

Sadhan Bhandari[1], Ram Prasad Kandel[2], Bimal Poudyal[1], Gopal Bhandari[1], Raghunandan Byanju[1], John M. Nesemann[3], Riju Shrestha[4], Valerie M. Stevens[3], Jason S. Melo[3], David A. Ramirez[5], Travis C. Porco[3], Kieran S. O'Brien[3], Thomas M. Lietman[3], Jeremy D. Keenan[3]

[1]*Bharatpur Eye Hospital, Bharatpur, Nepal,* [2]*Seva Foundation, Bharatpur, Nepal,* [3]*Francis I. Proctor Foundation, University of California San Francisco, San Francisco, CA, United States,* [4]*Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States,* [5]*Department of Ophthalmology & Visual Sciences, University of Iowa, Iowa City, IA, United States*

Local medical shops are often the de facto medical provider for treatment of eye conditions in Nepal, though workers are not required to have specific ophthalmic training. Steroid eye drops are commonly dispensed, but these medications can potentially worsen corneal abrasions and infections. To investigate the knowledge and practice patterns of medical shop workers in Nepal, all medical shops from a set of communities in the Chitwan and Nawalpur districts of Nepal were identified for a cross-sectional study. An employee from each shop was shown 4 corneal photographs (i.e., corneal abrasion, corneal infection, conjunctivitis, and no disease), and questions were asked regarding diagnosis and management for each. Among 117 participants, 86 (74%) identified conjunctivitis correctly but fewer were able to identify a corneal abrasion (50/117; 43%) or corneal infection (47/117; 40%). When asked a free-response question about how they would treat each condition, most workers answered they would prescribe a topical antibiotic for the corneal abrasion (95/117; 81%) or corneal infection (94/117; 80%), and few responded that they would prescribe a topical steroid (8 [7%] and 6 [5%], respectively). However, when given a multiple choice question, 15 (13%) respondents reported being willing to prescribe a topical steroid for a corneal abrasion, and 25 (21%) for a corneal infection. When a steroid was prescribed for corneal infection or abrasion, it was almost always a combination eye drop containing both a steroid and antibiotic. When asked if they would give any other recommendations, most workers responded that they would counsel clients to visit an eye care specialist, with the highest proportion giving this assessment for corneal infection (91/117; 78%) and corneal abrasion (81/117; 69% of respondents). Thus, in this area of Nepal, where local medical shop owners likely play a significant role in treating eye disease, most medical shop workers reported using an antibiotic for corneal abrasions and infections. Workers should be educated about the danger of using topical steroids in cases of suspected corneal infection.

41

PLASMA KYNURENINE TO TRYPTOPHAN RATIO IS NEGATIVELY ASSOCIATED WITH LINEAR GROWTH OF CHILDREN LIVING IN A SLUM OF BANGLADESH: RESULTS FROM A COMMUNITY-BASED INTERVENTION STUDY

Md Amran Gazi, Subhasish Das, Md. Abdullah Siddique, Md. Ashraful Alam, Shah Mohammad Fahim, Md. Mehedi Hasan, Farzana Hossaini, Md. Mamun Kabir, Zannatun Noor, Rashidul Haque, Mustafa Mahfuz, Tahmeed Ahmed

icddrb, Dhaka, Bangladesh

Chronic exposure to infectious agents results in environmental enteric dysfunction (EED) - a significant contributor to childhood stunting. Low plasma tryptophan (TRP), increased kynurenine (KYN) and KYN-to-TRP (KT) ratio are associated with infections and chronic immune activation. We postulated that both these conditions are interlinked, and therefore, aimed to identify association between KT ratio and the linear growth of Bangladeshi children. A total of 480 stunted and at risk of being stunted children aged 12-18 months were enrolled and provided nutrition intervention for 90 days. Plasma samples were assessed to measure TRP and KYN concentrations employing LC-MS/MS. Multivariable linear regression with generalized estimating equations (GEE) was applied to analyze association between the KT ratio and linear growth. TRP, KYN and KT ratio were significantly higher in stunted children compared to children at-risk of being stunted both at baseline and at the end of nutrition intervention. Following the intervention, KYN concentration was significantly reduced from 4.6 (3.6, 5.4) µmol/L to 3.9 (0.3, 7.6) µmol/L and KT ratio decreased from 104 (80.9, 131) to 92.8 (6.6, 247) in stunted children. We also found KT ratio to be negatively associated (coefficient =-0.7; 95% CI =-1.13, -0.26; p-value = 0.002) with linear growth. Additionally, KYN and KT ratio were positively correlated with fecal neopterin and plasma C-reactive protein while TRP was negatively correlated with both of these biomarkers and alpha-1-acid glycoprotein. Our findings imply that KT ratio is involved in the pathophysiology of stunting as well as with biomarkers of inflammation in Bangladeshi children.

42

TRANSLATING JAMAICAN PRIMARY AND PREVENTIVE CARE FROM POLICY TO PRACTICE

Dr. Lena S. Samuel, MD

New York University, New City, NY, United States

Translating Jamaican Primary and Preventive Care from Policy to Practice As a PAHO member country, Jamaica Is one of the largest English speaking Caribbean countries. With its population of 2.89 million, many go without Primary and Preventive Medical Care and often suffer from preventable non communicable and communicable diseases. Medical care is often not translated to every member of the community in Jamaica. Quality, consistent medical care is to be made available to all Jamaicans across the island including the Parishes of Kingston, St. Andrew, St. Catherine, Clarendon, Manchester, St. Elizabeth, Westmoreland, Hanover, St. James, Trelawny, St. Ann, St. Mary, Portland and St. Thomas. The aim is to decrease island wide preventable morbidity from emergency services, maternal morbidity/mortality, and communicable, non communicable diseases through primary care initiatives and guidelines for a nationwide primary care curriculum and electronic health record. While many local medicine and international medical missions come to provide primary care, continuity needs improvement across the island. Further mission organizations who present to the country to provide medical care will be subject to nationwide primary care medical practice regulations.

43

CENTERS OF EXCELLENCE IN SOUTHERN AFRICA; THE CASE OF MANHICA HEALTH RESEARCH CENTER, MOZAMBIQUE

Teresa Eduarda Machai

Manhica Health Research Center, Maputo, Mozambique

Trials of Excellence in Southern Africa - TESA Network of Excellence (NOE) was established with funding from the European Union through the EDCTP during EDCTP phase 1 with the aim of creating a structure for collaboration, capacity building and training of researchers as well as to increase the collaboration of the North-South and South-South networks among the 9 research institutions in 6 southern African countries. In 2017, the network was expanded and now is composed of 13 institutions from 8 countries in Southern Africa (SA) and 3 from Europe which came together to create and strengthen research capacities in SA. These members include Botswana Harvard AIDS Institute Partnership , Blantyre Health Research and Training Trust, Manhiça Health Research Center-CISM, KwaZulu-Natal Research Institute, Stellenbosch University, University of Cape Town, University of Zambia, Biomedical Research and Training Institute; University

of Zimbabwe College of Health Sciences, Centro de Investigação em Saúde de Angola (CISA), and the University of Namibia. To achieve its aim, the TESA consortium focus on strengthening institutional capacities, promoting professional development and scientific leadership fostering collaborations to maximize impact. TESA develops specific activities aiming to increase researchers critical mass devoted to health, develop first class institutions engaging clinical trials with the highest standards on the infectious diseases responsible for the most morbidity and mortality. The network is coordinated by the Director of the CISM who also happens to be a board member of the EDCTP. The fact of being a board member of the EDCTP enables him to advocate for expanded networking, training, capacity building "Hands on", and project management as well as to enhance collaboration with government agencies during his visits in the TESA network member states.The coordinator made an effort for additional funds out of TESA, and joint meetings within Ref. Labs for SWOT analysis. This approach has been appreciated by external auditors that recommended to other NOE in Western, East and Central Africa (WANETAN, EACCR, and CANTAM).

44

THE PRIVATE SECTOR AS A POTENTIAL DATA SOURCE FOR EPIDEMIOLOGICAL SURVEILLANCE AND CONTROL OF ANTIMICROBIAL RESISTANCE IN UGANDA

Anthony Kabanza Mbonye[1], Henry Kajumbula[1], Agnes Kiragga[2], Grace Banturaki[2], Patrick Sesaazi[2], Kenneth Katimbo[2], Ibrahimm Mugerwa[3], Kristian Hansen[4], Pascal Magnussen[4], Sian Clarke[5]

[1]*College of Health Sciences, Makerere University, Kampala, Uganda,* [2]*Infectious Diseases Institute, College of Health Sciences, Makerere University, Kampala, Uganda,* [3]*Ministry of Health, Kampala, Uganda,* [4]*University of Copenhagen, Copenhagen, Denmark,* [5]*LSHTM, London, United Kingdom*

Antimicrobial resistance is now considered to be among one of the biggest threats to global health. Poor practices amongst providers can hasten the emergence and spread of antimicrobial resistance (AMR). The objectives of the study are to assess the feasibility of AMR data collection in the private sector; to assess volume and type of antibiotics prescribed; the dosage and the conditions/illnesses for which antibiotics are prescribed; to assess drug sensitivities and drug resistance patterns; and to assess the acceptability of the data collection at private facilities. A pilot of data collection at private facilities using Online Data Kit (ODK), targets human private clinics, animal shops/clinics, pharmacies and private laboratories. Installation of the study database onto the national server is completed and this will allow collation and possible comparison of study findings with data generated from public health facilities. A total of 20 facilities are implementing the study; and data collection is planned for 6-9 months. The pprimary outcomes are: the feasibility of AMR data collection in the private sector measured by number of health workers trained in AMR data collection, number of records entered for i) private health workers, ii) clients exit surveys in private pharmacies, iii) number of exit clients at veterinary shops; the volume and type of antibiotics prescribed; the dosage and the conditions/illnesses for which antibiotics are prescribed; a and antibiotic drug sensitivity patterns. The field observations show; that there is a high response rate from facility owners and participants. They are happy about the intervention and are very enthusiastic; especially participants from veterinary drug shops seem to be more enthusiastic because nobody has ever involved them in a study. In the first month of study implementation, the research team is providing supervision to ensure adequate skills of participants and the timelines of data sharing with the national central repository. In conclusion, analyses of an integrated data set from the private, public, human and animal sectors will contribute to effective control of AMR in Uganda.1

46

PROMISING APPROACHES FOR IMPROVING PROVIDER ADHERENCE TO MALARIA TESTS: RESULTS FROM A BEHAVIORAL ECONOMICS PILOT IN NIGERIA

Angela Acosta[1], Faraz Haqqi[2], Sriram Sridharan[3], Temitope Ogunbi[4], Eno'bong Idiong[4], Idowu Akanmu[4], Faramade Alalade[4], Linda Osaji[4], Lucy Okolo[4], Ernest Obaseki[4], Folake Odubore[4], Justin DeNormandie[4], Jose Tchofa[5], Uwem Inyang[5], Foyeke Oyedokun-Adebagbo[5], Owoya Samuel[6], Nnenna Ogbulafor[6], Ian Tweedie[4], Bolatito Aiyenigba[4]

[1]*Breakthrough ACTION, Johns Hopkins Center for Communication Programs, Baltimore, MD, United States,* [2]*Breakthrough ACTION, ideas42, Washington, DC, United States,* [3]*Breakthrough ACTION, ideas42, New York, NY, United States,* [4]*Breakthrough ACTION, Johns Hopkins Center for Communication Programs, Abuja, Nigeria,* [5]*President's Malaria Initiative and United States Agency for International Development, Abuja, Nigeria,* [6]*National Malaria Elimination Program, Federal Ministry of Health, Abuja, Nigeria*

Presumptive treatment is a major challenge in Nigeria. Studies from Ebonyi and Ogun states show many public (51%) and private (95%) providers prescribe ACTs to test-negative patients, as reported previously. The 2018 DHS found that 42% of women and 54% of men would seek malaria treatment even if they had a negative test. In 2019, Breakthrough ACTION applied human-centered design and behavioral economics approaches to design and test interventions. A 3-month feasibility pilot ran in 9 primary health centers (PHCs) and 3 hospitals in 3 states (Kebbi, Nasarawa and Akwa Ibom). The package included: group discussions to address providers' misconceptions about rapid diagnostic tests, testing febrile cases at triage ("testing before consultation" [TBC]), a simplified pediatric evaluation form based on integrated management of childhood illness (IMCI), client-facing health talks and counseling, a performance tracking poster and supervision visits. Data sources included laboratory and outpatient register review, client exit interviews (N=65), pre-post provider surveys (N=207 & 137), and lessons learned/group discussions with providers (N=152) and government supervisors (N=34). Collection of baseline register data was not possible due to poor record-keeping, though HMIS and LMIS data from the same period in 2018 showed facility adherence ratios ranging from 4 to 66 test-positive malaria cases for every 100 ACTs dispensed. At month 1, only 2 facilities achieved the target range of 90-100 (variation: 9 to 188). By month 3, all PHCs had done so (variation: 91 to 110) while hospitals were had more clients testing positive without getting treatment (variation: 42 to 122). Knowledge and attitudes improved; the percentage of providers stating their peers trust negative malaria test results increased from 61-76%. Providers reported high satisfaction with TBC and the pediatric evaluation form and poor understanding of the performance-tracking poster. However, supervisors found the poster useful. Modifications for health talks were also identified. Results suggest the package shows promise. Revisions are underway for larger-scale deployment.

47

THE BURDEN OF SEXUAL DEVELOPMENTAL DISORDERS AMONG CHILDREN REPORTING TO A TEACHING HOSPITAL IN GHANA

Phans Oduro Sarpong[1], Francis Adjei Osei[2], Bright Atta Dankwa[3], Samuel Frimpong Odoom[4], Rita Ackah[5]

[1]*Kumasi Centre for Collaborative Research in Tropical Medicine, KCCR, Kumasi, Ghana,* [2]*Komfo Anokye Teaching Hospital, Pubic Health Unit, Kumasi, Ghana,* [3]*Ghana Institute of Management and Public Administration, School of Public Service and Governance, Kumasi, Ghana,* [4]*Child Health Directorate, Komfo Anokye Teaching Hospital, Kumasi, Ghana,* [5]*Tafo Government Hospital, Counselling Unit, Kumasi, Ghana*

Disorders of Sexual Development (DSDs), although rare, causes social disturbances to children, parents and the family at large. There are limited studies in this subject in Ghana thereby affecting its inclusion in

national policies. This present study sought to evaluate the extent of this phenomenon and the common presentation in Ghana. The study was conducted in the paediatric department of the Komfo Anokye Teaching Hospital (KATH). A retrospective cohort study was done through patient chart reviews from January 2011 to August 2015. Permission to publish the data was sought from the relevant authorities including the local ethics committee. A total of 51 DSD cases were recorded at the study site in the period under review. This accounts for an average of about 13 cases seen annually. More than half of the patients were within 12 months of age. The male to female ratio was proportionate. Ambiguous genitalia were the commonest sexual development disorder with 17 cases (36.96%) recorded. The next commonest was imperforated hymen also referred to as fused labia contributing 17.39% (n=8/51). Undescended testis, Partial Androgen Insensitivity, Congenital Adrenal Hyperplasia were among the other DSDs recorded. Among the males, a mean (SD) testosterone of 1.83 (±1.76) were recorded with a corresponding level of 0.85 (±0.64) found in females. For urea, an average level of 3.42 (±2.04) was recorded in males with 3 (±0.66) recorded in females. The study has brought to light one of the neglected but debilitating medical conditions affecting child development. A national surveillance to document this disability is needed in order to generate empirical evidence needed to trigger policies and programs that could potentially minimize this psychosocial challenge.

48

BARRIERS AND FACILITATORS OF FAMILY PLANNING USE IN FISHING COMMUNITIES OF LAKE VICTORIA IN UGANDA

Annet Nanvubya[1], Rhoda K. Wanyenze[2], Jean-Pierre Van geertruyden[3]

[1]*UVRI-IAVI HIV Vaccine Program, Entebbe, Uganda,* [2]*School of Public Health College of Health Sciences, Makerere University, Kampala, Uganda,* [3]*Global Health Institute, University of Antwerp, Antwerp, Belgium*

Family Planning (FP) allows people to manage their family sizes and is a key element in the conduct of HIV prevention research. Although fishing communities (FCs) are targeted populations for HIV prevention research, facilitators of FP use and barriers in these communities are poorly understood. We explored barriers of FP use and facilitators in FCs on L. Victoria in Uganda. We employed a mixed methods approach comprising a baseline cross-sectional survey, ten in-depth interviews and four Focus Group Discussions in two FCs. We used multivariable logistic regression to analyse quantitative data and a thematic approach to generate themes in the qualitative data. A total of 1410 individuals participated in the baseline survey and 47 in the qualitative component of the study. Just over a third (35.6%) were using FP with the most commonly used methods being condoms, pills and injectables. In Kigungu, participants whose religion was Anglican and Muslim were more likely to use FP compared to those whose religion was catholic ((aOR: 1.45 95% CI: 1.05-1.99) and (aOR: 1.45 95% CI 1.05-2.07) respectively). Participants were more likely to use FP if they had satisfactory FP knowledge compared to those with no satisfactory FP knowledge (aOR: 1.79 95% CI: 1.23-2.61), or if they were married as compared to their single counterparts (aOR: 1.84 95% CI: 1.32-2.57). Participants were more likely to use FP if they were having two or more sexual partners in the last 12 months as compared to those with less than two sexual partners (aOR: 1.41 95%CI: 1.07-1.87). In Nsazi, participants who reported having multiple sexual partners in the last 12 months were more likely to use FP as compared to those with less than two sexual partners (aOR: 2.60 95% CI: 1.36-4.97). Barriers to FP use included fear of side effects like infertility and excessive bleeding; fertility desire; gender preferences; method stock outs and lack of trained staff to offer surgical methods. FP use in FCs is sub-optimal. Barriers of FP use and facilitators are mainly religious, cultural, social and biomedical. There is a substantial need for FP education and strengthening of FP service provision in FCs.

49

SOCIAL AND CULTURAL DETERMINANTS THAT AFFECT KNOWLEDGE, ATTITUDES, AND PRACTICES OF MATERNAL HEALTH CARE UTILIZATION IN RURAL AND URBAN AREAS OF MYSORE, INDIA

Sara Richards[1], Praveen Kulkarni[2], Nayanabai Shabadi[2], David Hill[1]

[1]*Frank H. Netter MD School of Medicine at Quinnipiac University, North Haven, CT, United States,* [2]*JSS Academy of Higher Education and Research, Mysuru, India*

The primary tier of India's healthcare system consists of Primary Health Centers each catering to a population of 30,000. Within this system there are women who act as accredited social health activists (ASHAs) monitoring pregnant women, accompanying them to their antenatal visits, and ensuring they are receiving appropriate care. These services have been instrumental in improving maternal health access across India and consequently reducing maternal mortality. This study aimed to determine the social and cultural driving forces behind maternal health knowledge, attitudes and practices in Mysore. Study participants were recruited from three Primary Health Centers consisting of two rural centers and one urban center. Participants included the women at the centers receiving antenatal and postnatal care at the time of visitation over a period of 6 weeks. Quantitative variables were assessed by cross-sectional analysis via a comprehensive survey to quantify maternal health knowledge, attitudes, and practice and generate numerical data. Qualitative variables were assessed by means of a focus group discussion to measure trends in thoughts and opinion surrounding available maternal healthcare services. Final analysis included 56 urban women and 36 rural women (n=92). There was found to be a significant statistical difference in overall knowledge (p-value 0.004) between urban and rural areas with rural participants having more overall knowledge than urban participants. There are significant correlations (p-value 0.01) across the knowledge, attitude and practice domains with a positive correlation between knowledge and practice, knowledge and attitude, and practice and attitude. The knowledge gap can be attributed to the prevalence of ASHAs in rural communities. ASHAs are trusted healthcare influencers, overseers, and educators. Incentivizing more women to become ASHAs in urban communities can help close this gap in knowledge between the two environments. Specific programs dedicated to broadening the education of ASHAs will help improve the knowledge of the community and in turn improve on practice.

50

A CLUSTER-RANDOMIZED TRIAL ON THE COMMUNITY-BASED PREVENTION OF CORNEAL ULCERS: THE VILLAGE-INTEGRATED EYE WORKER TRIAL (VIEW)

Gopal Bhandari[1], Kieran S. O'Brien[2], Raghunandan Byanju[1], Ram Prasad Kandel[3], Bimal Poudyal[1], Mariya Gautam[1], John A. Gonzales[2], Travis C. Porco[2], John P. Whitcher[2], M. Srinivasan[4], Madan Upadhyay[5], Thomas M. Lietman[2], Jeremy D. Keenan[2]

[1]*Bharatpur Eye Hospital, Bharatpur, Nepal,* [2]*Francis I. Proctor Foundation, University of California San Francisco, San Francisco, CA, United States,* [3]*Seva Foundation, Bharatpur, Nepal,* [4]*Aravind Eye Care System, Madurai, India,* [5]*BP Eye Foundation, Children's Hospital for Eye, Ear, and Rehabilitation Services (CHEERS), Kathmandu, Nepal*

The Village Integrated Eye Worker trial was a cluster-randomized trial performed from 2014-2017 in Nepal to determine whether a community health worker program can reduce the incidence of corneal opacity. 24 rural village development committees (VDCs) in the catchment area of the Bharatpur Eye Hospital in the Nawalparasi and Chitwan districts were randomized in a 1:1 ratio to receive a community-level intervention or no intervention. In intervention VDCs, female community health volunteers (FCHVs) were trained to diagnose corneal abrasions and provide antimicrobial ointments and/or referrals as needed. A publicity campaign was conducted in the intervention VDCs to encourage residents to present to FCHVs within 24 hours of experiencing ocular trauma. The pre-specified

primary outcome was photographic evidence of corneal opacity, assessed by masked graders from corneal photographs taken of both eyes during door-to-door censuses at months 0, 12, 24, and 36. A total of 213,697 individuals were included on the baseline census and 486,063 person-years were monitored over the 3-year study period. The FCHV program was monitored in the 12 intervention VDCs to ensure treatment fidelity. Over the course of the 3-year program, FCHVs completed 11,350 initial visits, of which 7,078 (94.8%) involved ocular trauma. Antimicrobial treatment was dispensed to 5,348 individuals. At the conclusion of the 3-year study period, 302 incident opacities were identified in the intervention arm (incidence 1.22 per 1000 person-years), compared with 270 (incidence 1.27 per 1000 person-years) in the control arm (incidence rate ratio 1.05, 95% CI 0.63 to 1.73). Thus, this randomized trial failed to find a significant reduction in corneal opacity after implementing a community health worker program.

51

IMMERSIVE TRAVEL IS OFTEN INTERRUPTED BY ILLNESS WITH MORE THAN HALF OF TRAVELERS VISITING LOCAL CLINICS

James McCarty[1], Mark Schneider[2], Sarah Odeh[3]

[1]*Stanford University, Stanford, CA, United States,* [2]*Emergent Biosolutions, Gaithersburg, MD, United States,* [3]*Emergent Biosolutions (Consultant), Gaithersburg, MD, United States*

Illness, while on holiday, may have a significant impact on one's travel, including lost time, experiences, and cost. One cause of illness while traveling is cholera, a preventable disease endemic in many countries around the world. Travelers from Australia, Canada, Germany, Italy, Portugal, Spain, Sweden, and the UK completed a survey on their travel and vaccination experiences over the past 3 years. Respondents must have visited a cholera-endemic country. Survey questions included reasons for travel, pre-travel preparation, illness while traveling, and vaccination experiences. A total of 256 travelers completed the survey. The most common reason for traveling and for selecting a destination was immersion into the local culture (90%). On average, respondents took 4 international trips greater than 3 days for vacation/holiday. Safety and health risks were important criteria for most respondents (80%). As such, preparation for trips usually began 4 months in advance, and included seeking information on health risks at their destination (64%), purchasing travel insurance (63%), being vaccinated (58%), purchasing medication (56%), and consulting a healthcare provider (55%). Despite the availability of travel health related vaccinations such as cholera, yellow fever and others, only 40% of travelers report being vaccinated prior to travel. Of respondents receiving vaccinations, the most common reasons were due to healthcare provider recommendations and to avoid preventable illness. When asked about the impact illness had on past travel, 55% of respondents became ill or travelled with someone who fell ill. Of those, 57% had travel plans interrupted, 53% visited a local clinic or hospital, and more than 35% missed pre-booked activities, stayed inside for extended periods of time, or were not able to immerse in the local culture. In conclusion, illness while traveling is common and may negatively impact travel plans, preventing full immersion in local cultures and from taking full advantage of their holiday. Travelers should consider vaccination prior to departure to avoid preventable diseases.

52

TRAINING THE TRAINER: EMPOWERING HEALTHCARE WORKERS TO TEACH ABOUT HEPATITIS B IN THEIR COMMUNITIES

Nasreen Syeda Quadri[1], Shemal Shah[2], Holly Rodin[3], Jose Debes[4]

[1]*University of Minnesota, Minneapolis, MN, United States,* [2]*Health Partners Regions Hospital, Minneapolis, MN, United States,* [3]*Analytic Center of Excellence, Hennepin Healthcare, Minneapolis, MN, United States,* [4]*University of Minnesota; Department of Gastroenterology and Hepatology, Hennepin Healthcare; Arusha Lutheran Medical Center, Minneapolis, MN, United States*

Hepatitis B infection (HBV) causes a high yet under-recognized disease burden in sub-Saharan Africa. Unlike other chronic infectious diseases (i.e. HIV), there is a lack of awareness about HBV in communities at large. We developed a workshop to empower healthcare workers (HCW) to increase awareness about HBV in their respective communities. The workshop was implemented in Iringa, southern Tanzania, in the setting of an annual conference with 130 multi-disciplinary attendees from several regions and contained high-yield information about HBV with a focus on skills for engaging the community. Teaching focused on simple messaging, repetition and easy to disseminate information. Four major health messages about HBV were offered; hepatitis B is common, silent, preventable and complications can be manageable. To evaluate the immediate effectiveness of the workshop, a 5-question survey focused on important techniques for HBV teaching was administered before and after. The cohort consisted of 25 nurses, 3 doctors, 2 pharmacists, and spanned over one-hour. All questions in the survey showed improvement in percentage of correct answers after the workshop. Likely due to small sample size, only two pre-post test questions showed statistical significance in the percentage of correct answers. Specifically, evaluating that HBV is silent (7% pre vs. 87% post; p=0.0001), and that repetition is key to promote awareness (63% pre vs. 100% post; p=0.0002). 100% of doctors, 60% of nurses and 50% of pharmacists self-reported knowing personal HBV serostatus. Even after correction for self-reported previous HBV knowledge, the differences remained significant for both of those questions (11% pre vs. 86% post, p<0.0001) and (63% pre vs. 100% post, p=0.009), respectively. Four healthcare workers corresponded with questions and updates about educational sessions in their setting after an email was sent to all workshop participants one month later. Our approach of HBV workshops aimed at teaching HCWs to share health education with a community can be easily disseminated and applied in most parts of sub-Saharan Arica to increase awareness of HBV.

53

GENDER DIFFERENCES IN ALL CAUSES AND CAUSE SPECIFIC OF MORTALITY AMONG OLDER PERSONS IN RURAL UGANDA: IMPLICATIONS TO THE HEALTH CARE SYSTEM

Silver Kawere[1], Isaac Ddumba[2], David Kasibante[1]

[1]*Makerere College of Health Science, Kampala, Uganda,* [2]*African Research Centre 4 Ageing & Dementia, Kampala, Uganda*

Gender disparities in mortalities among older persons have been documented mostly in developed countries. However, information on gender difference on all causes and cause-specific mortality is lacking in developing countries. This study aims to describe the gender disparity in mortality rates in advanced age and their implication for the health systems in the rural SSA context. We used data on deaths that occurred among older persons aged 50 years plus, between 1st January 2006 to 31st December 2016. The Health Demographic Surveillance Site, is a longitudinal platform being implemented in Iganga district. Data analysis were performed using STATA 14. Death was classified using WHO InterVA, International Classification of Diseases (ICD-10). Deaths was categorized into four main thematic areas; Communicable diseases, Non-communicable diseases, external causes and unspecified causes. 1,513 deaths among older persons were recorded. A crude all-cause mortality rate of 6.55 per1000 (95% CI: 6.42-7.62) was seen during this time period. Across all gender, mortality from non-communicable diseases (NCDs) ranks highest among older persons with 59.6% compared to 28.5% from communicable disease. However, more female than men presented with NCDs, while more men than female presented with Communicable diseases. The all-cause mortality increased substantially (risk ratio 1.5(95% CI: 1.44-1.60) P<0.0001) due to a fourfold rise in deaths due to NCDs across age categories and sex (4.04 (95% CI: 3.98-5.34) P<0.0001). However, there is a significant difference in the risk of death across sex. The burden of older persons requiring chronic care has

substantially increased compared with those requiring acute care (1.68 (1.38-2.02): P< 0.0001 vs 0.6 (0.53-1.53) P=0.0002). In conclusion, there is a disproportionate increase in number of deaths resulting from NCDs in advanced age compared to communicable diseases in rural Uganda. This finding has implications for the delivery of health care for seniors, with integration of chronic care management in advanced age desirable to address the escalating burden.

54

ENGAGING LOCAL GOVERNMENT AUTHORITIES OFFICIALS AND BUILDING CAPACITY OF HEALTH FACILITY DATA OFFICERS IMPROVES DATA QUALITY, REPORTING AND TIMELINESS IN KWARA STATE, NIGERIA

Chinedu Chukwu[1], Linda Lawrence[1], Mariah Boyd-Boffa[2], Tom Hall[3], Isaac Adejo[1], Sonachi Ezeiru[4], Frank Oronsaye[4], Diwe Ekweremadu[4], Perpetua Uhomoibhi[5], Bala Mohammed Audu[5], Ibrahim Maikore[5], Cyril Ademu[5], James Ssekitooleko[6]

[1]*Management Sciences for Health (MSH), Abuja, Nigeria,* [2]*Management Sciences for Health (MSH), Medford, MA, United States,* [3]*Management Sciences for Health (MSH), Arlington, VA, United States,* [4]*Catholic Relief Services (CRS), Abuja, Nigeria,* [5]*National Malaria Elimination Program (NMEP), Abuja, Nigeria,* [6]*The Global Fund, Geneva, Switzerland*

Data is an essential element of any well-functioning health system. If officials are to make informed decisions about system performance they need to have quality data that is reported in a timely manner. Likewise, health facilities need to have staff available who are reporting quality data on a routine basis, and are available to make adjustments that improve service delivery based on feedback from higher officials. Management Sciences for Health, in collaboration with the National Malaria Elimination Program and Catholic Relief Services, with resources provided by the Global Fund Malaria Grant (2018-20), used a capacity building and advocacy approach to improve data quality (no errors for correctness, completeness and validity), reporting (within a 90-day window) and timeliness (by the 14th of each month) among 500 health facilities in Kwara State, Nigeria. The approach included data validation meetings to identify data issues using the facility Monthly Summary Forms and registers; onsite supervision by the 16 LGA data officers, who were trained on mentoring, coordination, setting expectations, and providing continuous feedback to health facility data entry officers, which included use of WhatsApp Groups and monthly bulk SMS to foster accountability; and, advocacy to encourage the LGA Primary Health Care Division to fully staff all facilities. The results from the Nigeria Health Management Information System show that data quality improved each year from 77% in 2017 (before the grant started) and 80% in 2018, to 90% in 2019. The data reporting rate improved from 78% in 2017 and 81% in 2018, to 96% in 2019, while reporting timeliness improved from 75% in 2017 and 78% in 2018, to 94% in 2019. These data demonstrate that engaging LGA officials and health facilities in a concerted effort, focusing on issues that affect data quality, reporting and timeliness, including staff and facility capacity, can improve key performance measures for data, creating an environment for improved decision-making and service delivery.

55

GENDER DIFFERENCES FOR UNINTENTIONAL FALLS AND RELATED INJURIES AMONG OLDER PERSONS IN UGANDA; PREVALENCE AND ASSOCIATED FACTORS

Doreen Nakibuuka[1], Isaac Ddumba[1], David Kasibante[2], Haruna Batange[3]

[1]*Makerere College of Health Science, Kampala, Uganda,* [2]*African Research Centre 4 Ageing & Dementia, Kampala, Uganda,* [3]*Community Health Access Foundation Uganda, Kampala, Uganda*

Falls are among the major causes of morbidity and mortality among older persons in later years. It estimated that, 20-30% of older persons that attend emergency outpatients will fall at least once. Injuries resulting from these falls posit a significant public health issues, particularly in later years. Hence, the study aimed to ascertain gender differences for unintentional and related injuries among older persons attending national emergency department. A cross sectional study unintentional and related injuries was conducted to 252 older persons. All patients above 60 who attended emergency department at national referral hospital were assessed. We asked about unintentional fall during the past one year. 202 older persons recalled having had at least one incident of falling in the past one year. 48.2% of older persons had had at least one incident of fall in a year preceding the interview. Of these 62.8% were women. 3 in 4 of the fall related injuries were due to laceration, fractures and abrasion. Women had more rates of injuries diagnosed, while, fractures were two folds (OR: 2.2 CI (1.92-3.84) common among men. Women were more 1.8 times (OR: 1.8 CI (1.56-4.23) more likely to be hospitalized than men. In conclusion, older women were disproportionately affected by unintentional falls related injuries than men. Although proven and effective fall prevention strategies exist, there is need to promote and contextualize these interventions in Uganda. Additional studies are needed to be conducted and determine gender differences to determine the risk factors for falls. The information is crucial in developing and implementing targeted fall preventions in Uganda.

56

A PRELIMINARY STUDY ON PERCEPTION OF THE COVID 19 PANDEMIC IN NORTHERN NIGERIA

Maryam Abdulrazaq Habib[1], Farouq Muhammad Daiyabu[2], Garba Iliyasu[3], Abdulrazaq Garba Habib[3]

[1]*Murtala Muhammad Specialist Hospital, Kano, Nigeria,* [2]*Infectious Disease Hospital, Kano, Nigeria,* [3]*Bayero University, Kano, Kano, Nigeria*

A pandemic of corona virus disease 2019 (covid19) emerged and affected most of the world in early 2020.To inform effective public health measures we conducted knowledge, attitude and practice survey in Kano state among a Hausa Muslim society in Nigeria in March 2020.Questionnaire was administered among urban 291(44.7%), periurban 52(8.0%) dwellers and to online 308(47.3%) participants.There were 651 study participants with median age 25 years, males 369(57%), 436(67%) had or are in tertiary education, compromising mostly students 301(46%) and civil servants(15%).The overall median (interquartile range) for knowledge, attitude and practice scores were 61%(18.58%), 76%(21.63%) and 62%(14.29%) respectively.Out of the respondents 169(26%) had good knowledge, 168(26%) had good attitude and 242(37%) had good practice using cut off scores 70%, 86.5% and 66.7% respectively. Over 57.3% did not agree covid19 originated from animals while 60.7% perceived the pandemic to be due to God's punishment. Most participants perceived no fear of covid19 though their attitude regarding social distancing measures showed 61% will decline attending wedding ceremonies, 70% agreed with cancellation of Muslim Hajj pilgrimage if the pandemic persists but 40% still insisted on attending Muslim Friday congregation prayer and 31.7% would still attend funeral rites. 28% felt some races are more at risk of the disease though 66% mentioned always practicing distancing themselves from persons coughing or sneezing. In univariate analysis increasing age and periurban habitation were associated with good knowledge while tertiary education was associated with good attitude [Odds Ratio;95% CI] 2.47(1.60-3.90). Similarly, those who were 'ever married' compared to singles had good knowledge 2.45(1.68-3.57). 67% always covered their mouth whilst coughing. Predictors of good knowledge in logistic regression included age 1.02(1.00-1.05), marital status 1.67(1.07-2.59) and education 0.68(0.56-0.82) while gender was not predictive. Knowledge of transmission and preventive measures should be improved in the population cognizant of cultural norms.

57

FORMATIVE RESEARCH TO UNDERSTAND CULTURALLY APPROPRIATE WAYS TO COLLECT POST-MORTEM TISSUE SAMPLES USING MINIMALLY INVASIVE TISSUE SAMPLING (MITS) FOR CHILDREN UNDER FIVE: AN OVERVIEW FROM ETHIOPIA

Ketema Degefa Begna[1], Berhanu Damise[1], Getahun Wakwaya[1], Adugna Tadesse[1], Nega Assefa[1], Lola Madrid[2]

[1]*Haramaya University, Ethiopia, Harar, Ethiopia,* [2]*London School of Hygiene & Tropical Medicine, London, United Kingdom*

The Child Health and Mortality Prevention Surveillance (CHAMPS) network aims to produce accurate information on the causes of child death based on data from pathology and microbiology using samples collected through Minimally Invasive Tissue Sampling (MITS), verbal autopsies and clinical data. In order to assess the feasibility of performing MITS, formative research is crucial to understand specific cultural, religious and socio-behavioral factors that may affect the acceptability of MITS. Data were collected in the regions of Kersa and Harar (eastern Ethiopia) from September 2017 to August 2019 through 12 in-depth key informant interviews, 6 focus group discussions, 6 semi-structured interviews, 22 participant observations and 6 photo-elicitation and rapid assessments and participatory walks (2) at Hiwot Fana hospital. Participants indicated that death is decided by God. They also explained that it is impossible to change the rule of religion and everything should be governed according to it. Participants also suggested touching the corpse is allowed only if the relatives of the dead child give consent and MITS should be done with consideration of the burial time. Support and incentives, such as accompanying grieving family while mourning, body transportation, grief gift, and a show of support from the MITS team attending the burial ceremony enhance acceptability. Discussions with Imams and Sheikhs to receive a fatwa supporting MITS and respecting beliefs attach death events to God's providence are the main issues to address when implementing MITS. Comforting the family before approaching for MITS consent, sharing emotions and grief, and follow-up and reciprocal feedback with religious leaders and community advisory boards are also recommended.

58

REASONS FOR REFUSAL OF POSTMORTEM MINIMALLY INVASIVE TISSUE SAMPLING IN THE DIAGNOSIS OF CAUSES OF CHILD DEATH IN KERSA, EASTERN ETHIOPIA

Eyoel Taye Bame[1], Ketema Degefa[1], Lola Madrid[2], Nega Assefa[1]

[1]*Haramaya University, Harar, Ethiopia,* [2]*London School of Hygiene & Tropical Medicine, London, United Kingdom*

Minimally Invasive Tissue sampling (MITS) is an alternative method to collect samples from dead babies in diagnosing the cause of death, however, the acceptance of this procedure is under question. In assessing the reasons for refusal of the MITS procedure, this analysis explored data from MITS-eligible cases the Child Health and Mortality Prevention Surveillance (CHAMPS) program in Kersa, Eastern Ethiopia. A retrospective document review was conducted on 48 MITS refusal cases. Observational ethnographic method was used during consent and family follow-up to record reasons for refusals. The study period was conducted for one year beginning in February 2019. The program registered 148 under-five death notifications in Kersa; of these, 68 families were approached for consent and only 24 families (35.3%) consented for MITS. The remaining 64.7% refused. The main reason for refusals are perceived poor health care while the child was sick resulting in the loss of trust in the health system. In addition, the distance from the MITS facility requiring a longer burial period, health care providers' behaviors, the unavailability of medications at the health facilities, and the unavailability of ambulance transport during the child's illness were mentioned as reasons for refusal. Acceptance of the MITS is highly influenced by the health care provided for the dead child during sickness. Respectful and compassionate care during sickness is important at all times; therefore, periodic appraisal of performance with the health authorities is essential. Efforts to build trust and address priority health problems through community engagement are also important.

59

EXPERIENCES OF ADOLESCENT PREGNANCY AMONG MAASAI IN KENYA: IMPLICATIONS FOR PREVENTION

Miriam A. Stats[1], David R. Hill[1], Josephine Ndirias[2]

[1]*Frank H. Netter M.D. School of Medicine, North Haven, CT, United States,* [2]*Mukogodo Girls Empowerment Program, Laikipia County, Kenya*

Adolescent fertility rates are high in Kenya and increase the likelihood of maternal and infant morbidly and mortality. Our objective was to (1) explore the prevalence of unintended pregnancy among Maasai adolescent mothers, (2) understand the context in which early pregnancy is occurring among the Maasai, and (3) investigate community-based strategies to prevent adolescent pregnancy within Maasai villages. In-depth individual interviews with 36 young Maasai women that gave birth during adolescence were conducted in Laikipia County, Kenya between June and July of 2019. Qualitative data was visualized using JMP software and coded using the framework method for content analysis. Several overarching themes were identified during this investigation. First, we found that pregnancy was unintended in 100% of cases. Second, we discovered that while 91% of participants described themselves as in a relationship at the time of conception, 75% self-identified as single by the time of delivery, and 81% identified as single by the present day. Of the participants that self-identified as in a relationship at the time of conception, 42% reported that the relationship ended because the father of the child denied responsibility for the pregnancy. When participants were asked what they wished could have happened differently, 37% responded that they wished they had never consented to be in the relationship that resulted in pregnancy. Another 33% of the participants responded that they wished they had never become pregnant due to its interference with their education. Our results suggest desire among our study population to prevent pregnancy and a significant unmet need for contraception.

60

ACCESS TO HEALTH CARE IN RURAL AREAS: CREATING A STRATEGY TO TRANSPORT SEVERELY ILL PEOPLE TO THE HEALTH FACILITIES WITHIN THE COMMUNITY IN SOUTHERN MOZAMBIQUE

Saquina Cossa[1], Maria Maixenchs[2], Felismina Tamele[1], Zubaida Manhenje[1], John Blevins[3], Quique Bassat[2], Inacio Mandomando[1], Khatia Munguambe[1]

[1]*Centro de Investigação em Saúde de Manhiça, Manhiça, Mozambique,* [2]*ISGlobal, Hospital Clinic, Universitat de Barcelona, Barcelona, Spain,* [3]*Emory Global Health Institute, CHAMPS Program Office, Emory University, Atlanta, GA, United States*

In low and middle income countries, access to health care in rural areas is difficult. Long distances, economic costs, lack of stock (drugs, supplies) and lack of personnel in health facilities have been identified as the main constrains. There is no doubt that this impacts health and survival, particularly among children. The Child Health and Mortality Prevention Surveillance (CHAMPS) program aims to increase knowledge on the cause of death of children under five in low and middle-income countries. CHAMPS includes a community engagement (CE) component aiming at involving the community, building partnerships and aligning healthcare and health seeking priorities. One recurrent issue during CHAMPS CE meetings was the lack of transport for the severely ill to health facilities. With the aim of sensitizing the community to engage in the design of a transport strategy, CHAMPS CE staff convened thirty-six meetings with community members and five meetings with possible drivers in 19 Neighbourhoods, involving a total of 847 participants, in Manhiça District (southern Mozambique). Six neighbourhoods agreed to co-design a transport strategy consisting of: i) the creation of an economic fund,

provided with the monthly contributions of each family that agreed to participate ii) designation of three community members responsible for managing the funds ii) identification and hiring of local "taxi drivers". The severity of the illness was decided to be evaluated by the community health workers living in each neighbourhood. Two months after implantation, 23 feedback meetings (40 participants) and 10 interviews with transportation system users were performed. Ten families benefited from transportation and all of them show satisfaction. Participants reported that the system was useful and functional, due to the continuous availability of transport and drivers' promptness. Engaging communities in designing their own strategy according to their local particularities and having the community manage their own initiative were crucial for the implementation.

61

DETERMINANTS OF HEALTHCARE SEEKING AND PROVIDER SELECTION: A CROSS-SECTIONAL STUDY IN RURAL HAITI

Molly Klarman[1], Justin Schon[1], Stace Maples[2], Youseline Cajusma[1], Valery M. Beau de Rochars[1], Chantale Baril[3], Eric J. Nelson[1]

[1]*The University of Florida, Gainesville, FL, United States,* [2]*Stanford University Libraries, Stanford, CA, United States,* [3]*Faculté de Medecine et de Pharmacie de l'Université d'Etat d'Haïti, Port au Prince, Haiti*

Identifying factors that influence care-seeking and provider selection are critical to develop desired, scalable and sustainable interventions to improve healthcare access. This is especially relevant in resource-limited settings. The objective of this study was to characterize household care-seeking behavior from formal and informal providers and their networks, in rural Haiti. A cross-sectional study was conducted among consenting households that had children less than 5 years of age. The sampling strategy was randomized by geographic location with stratifications for population density (low=0-111, medium=112-400, high=401-4665 households/2sq km). Household questionnaires contained a self-recall of health events during the previous month and two pediatric standardized cases; the goal was to characterize behaviors and intentions, respectively. Providers identified by households were characterized using a questionnaire that assessed practice type and clinical approach. The connectedness of households and their providers was determined by one-mode and two-mode network analysis. A total of 568 households and 65 providers were enrolled; 35% (n=220/636) of health events resulted in care-seeking. Perceived illness severity ('serious or life threatening') was associated with care-seeking (aOR= 3.17; 95%CI=1.96-5.13). There was a statistically significant difference between the intention to seek care from formal providers and the behavior of seeking care from formal providers (n=217, 34.76 chi-squared statistic, p<0.001). Households were less likely to seek care from informal providers as a function of increasing distance (aOR= 0.71; 95%CI = 0.53-0.93). The findings support a model that households are unlikely to seek care outside the home, yet when care is sought it is more often from nearby disconnected informal providers. Efforts need to be taken to reduce barriers to seek care from connected formal providers that may be more likely to practice accepted standards-of-care and/or develop methods to connect disconnected informal providers to conventional networks.

62

IMPACT OF CLIMATE CHANGE ON COVID-19 PANDEMIC: A SYSTEMATIC REVIEW

Muhammed O. Afolabi[1], Jennyfer R. Ambe[2]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*Capella University, Minneapolis, MN, United States*

The unpredictable outbreaks of viral diseases as witnessed during COVID-19 pandemic has necessitated the need to develop effective strategies to control human activities such as increase in international travel, deforestation and changes in social conditions induced by climate change. Little empirical evidence currently exists on the link between climate change crisis and COVID-19 pandemic. A systematic review was conducted to examine available evidence on possible association between climate change and COVID-19 pandemic. We searched Medline, Embase and Global Health databases for peer-reviewed articles reporting the impact of climate change on COVID-19 pandemic from database inception until 17 April 2020, using the search terms [(climat* OR climate change) AND (public health emergency OR pandemics OR disease outbreaks OR epidemics) AND (Coronavirus OR COVID-19 OR SARS-Cov-2 OR novel coronavirus)]. No language restrictions were applied. Grey literature was also searched. The search yielded 160 citations. After screening of titles/abstracts and de-duplication, three relevant publications were identified and included in the final narrative analysis. PRISMA guidelines were followed in conducting the review. Specific climatic conditions could serve as predictors of a peak of SARS onset. These climatic parameters may represent a direct outcome of the biological interactions between SARS-CoV and humans. The irreversible change in climate resulting in global warming provides conducive environment for disease-carrying insects to thrive, making outbreaks of viral diseases of global proportion to become recurrent. Also, human activities encroaching on habitats of animals has potential to contribute to this. In conclusion, limited evidence exists on the association between climate change and COVID-19. As a mark of global health response and preparedness for pandemic management, a need exists for developing robust research agenda to better understand the impact of climate change on disease outbreaks. This may engender an integrated approach to management of climate change and epidemics.

64

POVERTY REDUCTION THROUGH SUSTAINABLE SCHOOLS: EFFECTS OF A SCHOOL-BASED INTERVENTION ON SCHOOL REVENUE, EDUCATION, AND HEALTH OUTCOMES

Stephanie R. Monticelli[1], Scott McIntosh[1], Amina P. Alio[1], Daphne N. Pariser[2]

[1]*University of Rochester, Rochester, NY, United States,* [2]*Humans for Education, Rochester, NY, United States*

Poverty is a global epidemic with 736 million people living in extreme poverty worldwide. Investments in education and health have been shown to be critical poverty-reducing factors, but long-term maintenance of the programs can be financially straining. This study describes a multi-component, school-based intervention consisting of a small business, WASH+, and scholarship program that aims to reduce poverty through creating a system whereby the school can become financially sustainable and offer quality education and healthcare. In this study, we conducted a pre- and post-evaluation of the intervention at one school in Kenya to assess its impact on participants. Data collection included the collection of financial, education, and health outcomes. Outcomes were compared in the pre- and post-intervention period and analyzed using paired t-tests or ANOVA tests. Questionnaires and structured interviews were conducted with pupils and parents to assess health knowledge, behavior, and impact on family income and outcomes were compared using Chi-square analysis. Our evaluation found significant positive impacts across all tested parameters. Compared to the pre-intervention period, school income, pupil enrollment, attendance, and grades were all improved in the post-intervention period. Additionally, we observed a significant reduction in WASH-related illness and hospitalization costs. We also found evidence that health knowledge and behavior were high for pupils and that pupils disseminated positive health behavior to parents of the programs. Moreover, we observed that the programs positively impacted parental income through reductions in hospital fees, school fees, and savings on pupil food. These results show promise for poverty reduction and suggest that multi-component and integrated interventions can be more effective in establishing a methodology for impoverished schools to become financially stable, leading to better education and health outcomes for both pupils and the wider community.

65

EXPANDING TRAINING CAPACITY AND ACCESSIBILITY FOR MINIMALLY INVASIVE TISSUE SAMPLING

Tia Paganelli[1], Lindsay Parlberg[2], Norman Goco[2], Roosecelis B. Martines[3], Jana Ritter[3], Sherif Zaki[3], Edwin Walong[4], Washington Owino Ochieng[4], Dennis Inyangala[4], Walter Barake[4], Cyrus Wachiury[4], Natalia Rakislova[5], Lorena Marimon[5], Melania Ferrando[5], Jaume Ordi[5]

[1]*RTI International, Seattle, NC, United States,* [2]*RTI International, Research Triangle Park, NC, United States,* [3]*US Centers for Disease Control and Prevention, Atlanta, GA, United States,* [4]*Department of Human Pathology, School of Medicine, The University of Nairobi, Nairobi, Kenya,* [5]*Barcelona Institute of Global Health (ISGlobal), Barcelona, Spain*

The Minimally Invasive Tissue Sampling (MITS) Surveillance Alliance is a global, multidisciplinary consortium funded by the Bill & Melinda Gates Foundation which aims to expand the use of MITS globally and improve the quality of global mortality data. A primary objective of the MITS Alliance is to expand MITS training capacity and increase MITS training accessibility and availability, especially in low and middle-income countries (LMICs). In early 2019, through leadership from the MITS Secretariat and with input from the MITS Alliance Executive Committee, a MITS Training Technical Working Group (TWG) was convened to establish a training hub capable of providing ongoing MITS training. The MITS Training TWG consisted of representatives from the US Centers for Disease Control and Prevention (CDC), Barcelona Institute for Global Health (ISGlobal) and RTI International (RTI). Prospective locations for the training hub were considered and following a site assessment visit in April 2019, Kenyatta National Hospital (KNH), the teaching hospital of the University of Nairobi (UoN) in Nairobi, Kenya was selected. The Training TWG adapted existing MITS sample collection curricula and added content to promote skills in effective facilitation and interprofessional education. In July 2019, members of the Training TWG implemented the curriculum as part of a training of the trainers (ToT) for pathologists and pathology technicians at KNH. Subsequently a cadre of master trainers capable of supporting ongoing training as part of a MITS training hub was established. Based on participant feedback and observations during the ToT, the Training TWG refined and adapted the curriculum and included the addition of asynchronous online learning modules. The revised curriculum was piloted in August 2019 and through trainer observations and participant feedback, was fine-tuned through an iterative process over the course of six subsequent trainings. To date more than 30 pathologists and pathology technicians from eight different countries have participated in MITS sample collection training as part of the MITS Alliance Training Hub.

67

COST STUDY ANALYSIS OF SLEEPING SICKNESS INTERVENTION PROGRAMMES: A SYSTEMATIC REVIEW

Xia Wang-Steverding, Kamran Khan, Jason Madan, Kat S. Rock

University of Warwick, Coventry, United Kingdom

Gambiense human African trypanosomiasis (gHAT; sleeping sickness), is an infectious disease caused by the parasite *Trypanosoma brucei gambiense* and transmitted from person to person by the tsetse vector. It threatens millions of people in sub-Saharan Africa - without treatment, gHAT patients can die within a few years.This systematic review focuses on collecting all costs related to control, detection, and treatment of gHAT to compare costs across different countries. The review seeks to find cost drivers and to feed into an economic evaluation model of elimination strategy which can lay the foundation for future impact and benefit analysis.We identified 877 abstracts/titles in databases and 34 by hand search in grey literature. After deduplication, 365 records were excluded. After two independent reviewers screened, they agreed to exclude further 445 records. 66 full text articles were assessed for inclusion. Of these, 40 articles were included for data extraction.Costs were collected in the reported currency, noting the year of the study and study location. If possible both unit costs and total cost of the intervention were collected. Studies that reported only unit costs or only total costs were still included and extracted.We discovered from our included articles that earliest cost results were published in 1949 and most cost studies (9) were conducted in 2015. The cost studies mainly focus on the screening interventions (approx 30%), diagnostics (approx 25%) then treatment (approx 20%) in the Democratic Republic Congo and Uganda. Our results highlight that costs have only be publically reported for some interventions and in some endemic regions. It demonstrates the challenges in conducting robust economic evaluations and need for further cost collection work to guide future intervention strategies.

68

GROUPMAPPERS: INTEGRATING GEOSPATIAL TECHNOLOGIES AND CROWDSOURCING TO MAP COMMUNITIES FOR HEALTH PLANNING IN SOUTHEAST BANGLADESH

Sazid Ibna Zaman[1], Ipsita Sinha[2], Mohammad Abdul Quader[3], MIM Farhad[4], Abdul Majed Sajib[3], Md. Sabbir Hasan[5], Muhib Kabir[6], Shakil Ahmed[7], Humaira Akter[8], MD. Nurullah[8], Afrida Asad[8], Mostafa Amir Faisal[8], Nazim Uddin Ahmed[9], Anjan Saha[10], Didar Uddin[1], Richard J. Maude[1]

[1]*Mahidol Oxford Tropical Medicine Research Unit (MORU), Bangkok, Thailand,* [2]*Nuffield Department of Medicine, University of Oxford, Oxford, UK., Oxford, United Kingdom,* [3]*Department of Geography & Environment, Jagannath University, DHAKA, Bangladesh,* [4]*Center for Geoservices and research, DHAKA, Bangladesh,* [5]*Bangladesh Agricultural Research Council, DHAKA, Bangladesh,* [6]*Department of Transportation Studies, Morgan State University, Baltimore, MD, United States,* [7]*BRAC James P Grant School of Public Health, DHAKA, Bangladesh,* [8]*Department of Geography & Environment, Dhaka University, DHAKA, Bangladesh,* [9]*Department of Geography, Chandpur College, Chandpur, Bangladesh,* [10]*National Malaria Elimination and Aedes Transmitted Disease Control Program, Communicable Disease Control, Directorate General of Health Service, DHAKA, Bangladesh*

Most countries lack current and complete data on the locations, boundaries, names and populations of communities since traditional methods of data collection are time consuming and costly. This can severely constrain disease surveillance, disaster relief and delivery of essential services including healthcare, nutrition, electricity and water. It can thus be a major threat to public health and a substantial barrier to achieving sustainable development goals. Bangladesh is typical of developing countries with this being particularly problematic in rural and remote areas. A simple, rapid and low-cost method was developed and applied by a team of volunteers and researchers to map communities in southeast Bangladesh by annotation of satellite images using Google Earth™, participatory mapping and field GPS data collection. The results were compared to the existing government village registry, GPS coordinates collected in the field and used to estimate population and measure distances to health facilities. 70 volunteers mapped polygon boundaries of clustered communities and point locations of dispersed settlements in southeast Bangladesh. Over four months, the team iteratively developed methods and mapped an area of 15,836 km^2 with annotation of 43,418 polygons and 142,022 points covering 4 districts (Bandarban, Khagracchari, Rangamati and Cox's Bazar) followed by validation against GPS coordinates collected in the field and naming the communities. Some example use cases of how the results are being used for health planning are presented including measuring the distance of communities from health facilities and estimating population size. A simple, robust and easily scalable method was developed and successfully applied by a group of volunteers to map communities in southeast Bangladesh. The results have immediate utility for the government for disease surveillance and health service provision. Work is ongoing to develop robust methods for collecting the names of the communities and to expand the mapping to other areas.

69

ORGANIZATIONAL LEARNING AS AN "OPERATING SYSTEM" FOR COMMUNITY AND STAKEHOLDER ENGAGEMENT: INSIGHTS FROM THE LYMPHATIC FILARIASIS ELIMINATION PROGRAM IN PORT-AU-PRINCE

Breanna K. Wodnik, Michelle A. Grek, Lee T. Wilkers, James V. Lavery

Emory University, Atlanta, GA, United States

A core function of community and stakeholder engagement (CSE) is to reveal insights about the way global health programs might affect the interests of various stakeholders. Knowledge of these interests can help programs understand the kinds of value they might create for stakeholders, but also where programs may run the risk of creating harm. The extent to which programs are able to act constructively on the insights arising from CSE activities is determined largely by their capacity for organizational learning. In our recent investigation of declining mass drug administration (MDA) coverage for lymphatic filariasis (LF) in urban Port-au-Prince, Haiti, we found a gap between the insights about stakeholder interests gained through CSE activities and the LF elimination program's ability to respond constructively to them. The World Health Organization's 2000 guidelines for national LF elimination programs state that "(t)he manual will be adapted and changed as the LF community "learns-by-doing"", but offers no further guidance about how this should be achieved. Drawing from the findings of our Port-au-Prince case study, we show how an understanding of stakeholder interests created opportunities for learning, dialogue, and deliberation within the program. Missed opportunities to learn effectively about stakeholder interests had significant costs for the LF MDA campaign in Port-au-Prince, including inaccurate accounts of the program as it was actually delivered; discouragement among program staff; negative aspects of the program becoming embedded or repeated in the campaign design; and compromised evaluation and accountability. Our findings demonstrate the role of organizational learning as the "operating system" to realize the value of CSE, and constitute a novel and important conceptual shift in understanding the function and significance of CSE in global health programs in a way that moves learning beyond the annual reporting of specific outcomes. Organizational learning allows CSE to shape and potentially transform how stakeholder interests matter ethically and practically in global health and public health programs.

70

KNOWLEDGE AND ATTITUDE TOWARDS SICKLE CELL DISEASE AND PRENATAL SCREENING AMONG WOMEN ATTENDING ANTENATAL CLINIC IN THE GAMBIA

Momodou E. Jallow, Modou Bella Jallow

Edward Francis Small Teaching Hospital, Banjul, Gambia

Haemoglobinopathies are the commonest genetic disease worldwide & includes disorders affecting the structure, function or production of hemoglobin. In Sickle Cell Disease (SCD), there is a substitution of glutamate with valine in position of 6 beta-globin. Africa is the most affected continent with 200,000 newborn affected by Sickle Cell Anemia per year. The purpose of the study was to determine the knowledge & attitude towards sickle cell disease and prenatal screening in women attending antenatal clinic at JFPH(Gambia). A prospective study was done among women attending antenatal clinic using questionnaire. Hundred pregnant women were included in the study through a non-probabilistic convenience sampling method. Study participants ages ranged from 17-38 years & most of the participants were between 26 to 30 years(39%). Majority (91%) participants were in their second or third trimester. Fifty eight (58%) have knowledge about existence SCD but comprehensive knowledge about the cause, clinical manifestation & prevention of SCD was very low. Only 9% were screen & only one participant knew her genotype AS. Family and friends were main source of knowledge about SCD accounting for 32%. Ninety five (95%) of the participants would accept prenatal screening despite 70% of the participants had low knowledge about prenatal screening. Comprehensive knowledge about SCD was low despite good awareness of the existence of the disease among respondents.Educational qualifications had a significance influence on the knowledge & attitude of the respondents. The attitude towards premarital & prenatal screening was good. However, the attitude towards selective abortion of affected fetus was poor & this is mainly due to religious influence on the respondents.

71

HOW MANY *LUTZOMYIA UMBRATILIS* (DIPTERA: PSYCHODIDAE) SPECIES ARE THERE?

Vera M. Scarpassa[1], Ronildo B. Alencar[2]

[1]*Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brazil,* [2]*Fundação de Vigilância em Saúde, Manaus, Amazonas, Brazil*

Lutzomyia umbratilis is believed to be the main vector for *Leishmania guyanensis* in the Brazilian Amazon and neighboring countries. This study investigated the molecular variation and phylogeographic structure of *L. umbratilis* populations collected from a broad geographic area in the Brazilian Amazon. Samples were collected from nine sites along the Amazonas/Solimões and Negro rivers and from interfluves. The COI and Cytb mitochondrial DNAs from 363 individuals were sequenced. Statistical analyses focused on population genetics, phylogenetic relationships and species delimitations. The dataset consisted of 176 COI and 187 Cytb sequences, with length of 1,181 and 512 bp, respectively. COI genetic diversity was very high, while Cytb diversity was moderate. COI genealogical haplotypes, structure population and phylogenetic analyses identified deep and significant genetic differentiation and the presence of three main genetics groups, whereas for Cytb, the analyses showed a shallower genetic structure with two main haplogroups and not well resolved phylogenetic trees. These findings combined with the absence of isolation by distance for both markers, support the hypothesis that the fluvial system formed by Amazonas/Solimões and Negro rivers and interfluves, are the main evolutionary forces driving *L. umbratilis* diversification. The three *L. umbratilis* main genetic groups may represent three lineages, possibly species. The first lineage is in the north Amazonas river, where *Le. guyanensis* transmission is intense, implying that *L. umbratilis* is an important vector there. The second lineage is in the south Amazonas river, previously reported to be free area of *Le. guyanensis* transmission. The third lineage, first recorded in this study, is in the interfluve Amazonas/Solimões and Madeira rivers and was the most divergent lineage. The involvement of the second and third lineages in leishmaniasis transmission remains to be elucidated and should be investigated further. Our findings will aid epidemiological studies, surveillance and vector control programs in these regions, where disease transmission can be very high.

72

FIRST MOLECULAR DETECTION OF *RICKETTSIA AFRICAE* AND *RICKETTSIA AESCHLIMANNII* IN TICKS COLLECTED FROM CATTLE LOCATED IN NORTHERN AND SOUTHERN REGIONS OF GHANA

Janice A. Tagoe[1], Shirley Nimo-Paintsil[2], Mba Mosore[1], Seth Offei Addo[1], Clara Yeboah[1], Bright Agbodzi[1], Eric Behene[1], Danielle Ladzekpo[1], Charlotte Addae[1], Courage Dafeamekpor[3], Victor Asoala[4], Langbong Bimi[5], Anne Fox[2], Chaselynn Watters[2], LCDR Terrel Sanders[2], LCDR David Wolfe[2], Christina Farris[6], CDR Andrew Letizia[7], LCDR Joseph W. Diclaro II[8], Samuel Dadzie[1]

[1]*Noguchi Memorial Institute for Medical Research, Accra, Ghana,* [2]*Naval Medical Research Unit No. 3, Ghana Detachment, Accra, Ghana,* [3]*Ghana Armed Forces, Veterinary Department, Accra, Ghana,* [4]*Navrongo Health Research Centre, Navrongo, Ghana,* [5]*University of Ghana, Department of Animal Biology and Conservation Sciences, Accra, Ghana,* [6]*Viral and Rickettsial Diseases, Naval Medical Research Centre, Silver Spring, MD,*

United States, [7]Infectious Diseases Directorate, Naval Medical Research Centre, Silver Spring, MD, United States, [8]Navy Entomology Centre for Excellence, Jacksonville, FL, United States

Ticks facilitate the spread of pathogens globally, thereby influencing the health of both humans and animals. Ticks have been documented to infest pets and livestock as well as transmit bacterial pathogens including *Rickettsia* species. Many Ghanaian households rear animals which increases the risk of infection. It is therefore important to identify the tick species present in Ghana and determine their potential to transmit diseases. In this study, 1,493 ticks were collected from livestock across selected sites in Northern and Southern Ghana from June 2016 to December 2018. Ticks were pooled and analyzed for *Rickettsia* species. Out of the 541 pools, *Rickettsia* DNA was detected in 308 of the pools (56.93%) with *Amblyomma variegatum* being the most commonly infected species (N = 252, 69.61%). Of the *Rickettsia* positive pools, 77.27% were positive for *Rickettsia africae*. *Hyalomma truncatum* ticks were found to have the highest overall *Rickettsia* infection rate of 76.0% (95% CI, 30.0-99.0). However, the positive rate of *R. africae* infected pools was displayed in *A. variegatum* ticks at 54.2% (95% CI, 47.8-60.7). Further sequencing of 12 *Rickettsia* positive pools revealed the presence of *Rickettsia aeschlimannii.* This study reports the first molecular detection of *R. africae* and *R. aeschlimannii* in tick species in Ghana. This study builds our knowledge on the presence of spotted fever group *Rickettsia* species in Ghana. Further studies are needed to determine the risk of potential spread in human populations within the studied regions.

73

TECH OR TRADITIONAL: A FIELD TESTING OF AERIAL DRONE TO SAMPLE TICKS

Mami Hitachi, Hiroki Hashizume, Suguru Taga, Naoyuki Yamada, Satoshi Kaneko, Kazuhiko Moji, Tomonori Hoshi

Nagsaki University, Nagasaki, Japan

Tick-borne diseases are drawing increased attention worldwide owing to the severe disease and social burden they cause, especially in developed countries. Conducting regular tick sampling requires effective sampling tools and is essential in order to better understand disease epidemiology and ecology. Currently available methods (e.g. the drag flag method) are prone to sampling bias due to variation in the skill of the operator. In addition, only a limited area can be surveyed due to challenges of accessibility and limited human resources. To overcome these challenges, aerial drones have the potential to be used for tick monitoring surveys. The cost of drone technology has greatly decreased in recent years and their performance has increased, and therefore they offer an exciting alternative tick sampling method. Drones have high maneuverability and can enable researchers to collect data autonomously from environments where there are high chances of being bitten by ticks and thus reducing operator exposure is critical. Using an aerial drone, we aim to create an improved method for sampling ticks. A compact drone (< 200g) equipped with a white flag of flannel fabric was developed and its sampling efficiency compared to traditional drag-flag methods. The experiments are underway in various environments in Nagasaki, Japan. The results of the field sampling comparison will be presented and we will discuss opportunities for use of the drone for conducting tick surveys.

74

POTENTIAL ENTOMOLOGICAL AND HUMAN FACTORS INFLUENCING RESIDUAL MALARIA TRANSMISSION IN SELECTED AREAS OF MYANMAR

Kyaw M. Tun[1], Khin Lin[1], Aung Thi[2], Khin T. Win[1], Thant Z. Aung[3], Saw Lwin[1], Nu Nu Khin[4], May A. Lin[1], Neeraj Kak[5], Hala J. AlMossawi[5]

[1]*University Research Co., LLC, Yangon, Myanmar,* [2]*National Malaria Control Programme, Ministry of Health and Sports, Myanmar, Nay Pyi Taw, Myanmar,* [3]*University Research Co., LLC, Toungup, Myanmar,* [4]*President's Malaria Initiative, USAID, Yangon, Myanmar,* [5]*University Research Co., LLC, Chevy Chase, MD, United States*

Myanmar aims to eliminate malaria by 2030. The annual parasite incidences (per 1,000 population) of Rakhine was significantly decreased from 37.7 in 2011 to 4.1 in 2018 and that of Tanintharyi was decreased from 36.9 in 2011 to 3.0 in 2018 with the international support from Global Fund and PMI/USAID. However, there are some areas where the number of malaria cases remains relatively high despite adequate distribution of long-lasting insecticidal nets (LLINs) and provision of malaria diagnosis and treatment services. In order to assess the characteristics of human behavior and local vectors in these focal areas, an entomological survey and focus group discussions were conducted from March to August 2019 in the selected 10 villages/worksites of Tanintharyi Region and Rakhine State of Myanmar. In the entomological survey, 11 Anopheles species were collected, including the primary vectors (*An. dirus*, *An. minimus)* and secondary vectors (*An. maculatus*, *An. culicifacies, An. sundaicus, etc.)*. Biting time was ranged from 6:00 PM to 12:00 PM, while peak biting time was between 8:00 PM to 11:00 PM during which 62% of bites took place. Regarding *Anopheles* larval collection, *An. minimus*, *An. maculatus* and *An. barbirostris* were collected from edges of streams, wells and cattle footprints. During outdoor collection, 3 *An. dirus (1.4%)* and 7 *An. minimus (3.2%)* were caught among all 218 Anopheles species collected. Outdoor biting rate ranged from 0.02 to 0.33 bites per person per night while no other vector was caught at indoor collection. The villagers disclosed that night-time forest workers used to take bath around 8:00 PM in the streams, after daily forest work activities. They also revealed that they had received adequate LLINs. It was also pointed out that the effectiveness of LLIN to prevent mosquito bite may be reduced by night time works and taking bath in the streams in the late evening close to peak mosquito biting. Alternative interventions such as larval source management, insecticide treated clothing, or repellents should be provided upon the findings of the local context for effective controlling of outdoor malaria transmission in Myanmar.

75

FIRST REPORT ON THE USE OF NEAR-INFRARED SPECTROSCOPY TO AGE-GRADE *PHLEBOTOMUS PAPATASI* SAND FLIES

Catherine M. Flanley[1], Taina N. Ferreira[2], Douglas A. Shoue[1], Floyd E. Dowell[3], Mary Ann McDowell[1]

[1]*University of Notre Dame, Notre Dame, IN, United States,* [2]*Fundação Oswaldo Cruz, Rio de Janeiro, Brazil,* [3]*United States Department of Agriculture, Manhattan, KS, United States*

Vector age and survival directly influence vectoral capacity and disease transmission potential. Current age-grading techniques for phlebotomine sand flies are time intensive and destructive as they require dissection by highly skilled laboratory personnel that groups hematophagous females as young or old depending on parity status. For the first time, this study demonstrates the potential of near-infrared spectroscopy (NIRS) to age-grade laboratory-reared *Phlebotomus papatasi* sand flies, incriminated vector of *Leishmania major*, a causative agent of cutaneous leishmaniasis. Female (n=746) and male (n=497) sand flies were collected and preserved in RNA*later* at 0 (less than 24 hours), 7, 14, and 21 days post-eclosion. Male sand flies did not survive to the 21-day time point. The spectra from individual sand flies were used to develop a partial least squares (PLS) regression model using a leave one out cross validation (CV). Independent test sets of *P. papatasi* were used to test the model's prediction accuracy. When categorized as <7 or ≥7 days old, the overall predictive accuracy was 85% for females and 87% for males. Sand flies reared in slightly cooler insectary conditions were also predicted with greater than 80% accuracy on average. This study validates the use of NIRS as a non-destructive and rapid age-grading technique for *P. papatasi.*

76

ASSESSING THE SUSCEPTIBILITY OF FLEA VECTORS TO INSECTICIDES AS PART OF PLAGUE RISK MONITORING IN MADAGASCAR, 2019

Mireille Harimalala, Soanandrasana Rahelinirina, Anja Andrianaja, Slash Rakotovao, Minoarisoa Rajerison, Romain Girod

Institut Pasteur, Antananarivo, Madagascar

The causative bacterium of plague, a public health concern in Madagascar, is transmitted from rodents to humans via flea bites. Insecticides are the primary response tool used to control fleas therefore; monitoring insecticide resistance is critical. We assessed the susceptibility of wild-caught flea populations to three insecticides. Live fleas (*Xenopsylla cheopis*) were collected from field-captured rodents from 15 sites in 5 districts (east, south and central highlands) and were reared in an insectary. Twenty seven assays were conducted on flea populations (60 fleas per population); 11 with fenitrothion (currently used), nine with permethrin (a possible alternate) and seven with deltamethrin (a previously used insecticide). Fleas were exposed to insecticide-impregnated paper in the following concentration/duration combinations: fenitrothion 1% for 5 hours, permethrin 0.75% and deltamethrin 0.05% for 8 hours each. Papers were removed and fleas were left on the lab bench for 24 hours before recording the mortality rate (MR). We used WHO guidelines to determine susceptibility. Flea populations were classified as either resistant (MR < 80%), tolerant (80% ≤ MR < 98%) or susceptible (98% ≤ MR ≤ 100%) to insecticide. Flea susceptibility varied by insecticide type, concentration and location of sampling. Overall, resistance was observed in 23 (85%), tolerance in one (4%) and susceptibility in three (11%) assays. Eight (73%), one (9%) and two (18%) populations were resistant, tolerant and susceptible to fenitrothion, respectively. Nine (100%) populations were resistant to permethrin. Six (86%) populations were resistant to deltamethrin and one (14%) was tolerant. Resistance to all insecticides was observed in four sites. Susceptibility to fenitrothion was detected in two sites. Flea populations exhibited resistance to multiple insecticides including fenitrothion, the currently used product. This may be related to insecticide use for plague control, and also with the unregulated use of these chemicals (e.g. agriculture). Fenitrothion may still be a useful tool in sites where fleas remain susceptible.

77

COMPARISON OF YEAST-ENCAPSULATED ESSENTIAL OILS FOR MOSQUITO POPULATION CONTROL

Alexandra V. Yingling, Tzion Castillo, Ivy Hurwitz

University of New Mexico Health Sciences Center, Albuquerque, NM, United States

Millions of people are affected each year by mosquito-borne diseases like dengue and chikungunya. As the death tolls from these diseases continue to rise, the need for mosquito population control becomes more urgent. Current mosquito larval source management (LSM) approaches include the use of entomopathogenic bacteria and insect growth regulators, which are fraught with issues of vector resistance and detrimental effects on both human and environmental health. In previous work, our laboratory has developed an alternative environmentally friendly larvicide shown to be efficacious in killing *Aedes aegypti* larvae. The larvicide simply consists of orange oil encapsulated into yeast cells. In this work, the encapsulation of other essential oils, namely clove bud and fennel, into yeast cells will be explored for larvicidal application. The development of multiple larvicides broadens the accessibility of this technology on a global scale, as the larvicide synthesis can be tailored to the local context. The chemical constituents and encapsulation efficiencies of the oils were determined via high performance liquid chromatography. The variations in chemical constituents of the oils and their relative mortality rates on *Ae. aegypti* larvae will be discussed. These results will demonstrate the potential for this larvicide technology as a cost-effective integrated LSM approach, which may contribute to global health improvement.

78

NO NEED TO WING IT: A NEW METHOD FOR QUICKLY AND ACCURATELY AGE-GRADING MOSQUITOES USING WING MORPHOLOGY

Lyndsey Gray[1], Bryce Asay[2], Emmanuel D. Sougué[3], Blue K. Edwards[1], Anyirekun F. Somé[3], Roch K. Dabiré[3], Sunil Parikh[4], Brain D. Foy[1]

[1]*Colorado Sate University, Fort Collins, CO, United States,* [2]*American Public Health Laboratories and Center for Disease Control and Prevention, Salt Lake City, UT, United States,* [3]*Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso, Burkina Faso,* [4]*Yale University, New Haven, CT, United States*

The average age of a mosquito population is one of the most important determinants of vectorial capacity. Still, researchers lack a fast and reliable method for identifying older female mosquitoes, the most efficient disease vectors, and estimating the age of wild mosquitoes. We investigate scale loss along posterior wing edges as a simple, accurate means of determining mosquito age. We conducted experiments with *Anopheles gambiae* reared in two separate mesocosms. One mesocosm was exposed to blood meals with a sub-lethal dose of ivermectin (IVM) to determine if population age structure shifts could be detected via wing scale counting. Females were sampled from both mesocosms to analyze dissected wings and ovaries. When using parity assessments, no significant difference in age structure could be found between the two mesocosms (X^2 0.21, p-value 0.65). Because sub-lethal IVM treatments hinder ovarian development, this suggested that parity status was not a reliable age assessment tool when mosquitoes were exposed to control options that interrupted reproductive cycles. Wing scale counting detected a significant difference in average number of wing scales between both mesocosms (t-test -3.95, p-value <0.001), signifying that it was a comparatively more accurate age-grading method. Early explorations with this data indicate mosquito age can be predicted with high accuracy using machine learning algorithms. We also conducted a preliminary analysis on wildtype *An. gambiae* collected during our phase III, double-blinded IVM trial in Burkina Faso. Mosquitoes were captured one and three weeks post-mass drug administration from households in clusters randomized to IVM or placebo. Mosquito age structure analysis for each cluster and time point was made using histograms and compared to survival bioassay data. Younger mosquito age structure and decreased mosquito survivorship roughly correlate in two clusters where the strongest mosquitocidal effect was observed. Overall, these findings suggest wing scale counting is a viable method for quantifying mosquito age and detecting age structure shifts in response to vector control interventions.

79

A PROTOCOL FOR A CLUSTER RANDOMIZED TRIAL OF ONE-DOSE VERSUS TWO-DOSE IVERMECTIN MASS DRUG ADMINISTRATION FOR SCABIES IN REMOTE ISLAND COMMUNITIES IN SOLOMON ISLANDS

Susanna J. Lake[1], Sophie L. Phelan[2], Daniel Engelman[1], Oliver Sokana[3], Titus Nasi[3], Dickson Boara[3], Christina Gorae[3], Tibor Schuster[4], Anneke C. Grobler[1], Millicent H. Osti[1], Ross Andrews[5], Michael Marks[6], Margot J. Whitfeld[7], Lucia Romani[2], John M. Kaldor[2], Andrew C. Steer[1]

[1]*Murdoch Children's Research Institute, Parkville, Australia,* [2]*Kirby Institute, University of New South Wales, Sydney, Australia,* [3]*Ministry of Health and Medical Services, Honiara, Solomon Islands,* [4]*McGill University, Montreal, QC, Canada,* [5]*Australian National University, Canberra, Australia,* [6]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [7]*St Vincent's Hospital, University of New South Wales, Sydney, Australia*

Scabies is a significant contributor to global morbidity. The parasitic skin disease is estimated to cause 455 million annual incident cases world-wide. Scabies is endemic in many resource-limited tropical settings, including the nation of Solomon Islands in the South Pacific, where the population prevalence of scabies is approximately 20%. Scabies infestation

not only causes intense itch and discomfort, it leads to the development of impetigo, severe bacterial infections and immune-mediated disease. Community-wide ivermectin-based mass drug administration (MDA) is an effective control strategy for scabies in island settings, with a single round of two-dose MDA reducing population prevalence by around 90%. However, current two-dose regimens present a number of barriers to programmatic implementation of MDA. We designed the Regimens of Ivermectin for Scabies Elimination (RISE) study to investigate whether one-dose MDA is as effective as two-dose MDA to control scabies in high-prevalence settings. RISE is a cluster randomised non-inferiority trial. The study will be conducted in 20 isolated villages in Western Province of Solomon Islands. Villages will be randomly allocated in a 1:1 ratio to receive either one-dose or two-dose ivermectin-based MDA. The primary objective of the study is to determine if ivermectin-based MDA with one dose is as effective as two doses in reducing the prevalence of scabies after 12 months. Secondary objectives include the effect of ivermectin-based MDA on impetigo prevalence after 12 months and 24 months, the prevalence of scabies at 24 months after the intervention, the impact on presentations to health facilities with scabies and impetigo, and the safety of one-dose and two-dose MDA. The results of this study will guide policy for the dosing regimens of ivermectin for scabies MDA in high-prevalence settings.

80

PREVALENCE OF SCABIES AND IMPETIGO IN SOLOMON ISLANDS

Susanna J. Lake[1], Daniel Engelman[1], Oliver Sokana[2], Titus Nasi[2], Dickson Boara[2], Anneke C. Grobler[1], Millicent H. Osti[1], Ross Andrews[3], Michael Marks[4], Margot Whitfeld[5], Lucia Romani[6], John M. Kaldor[6], Andrew C. Steer[1]

[1]*Murdoch Children's Research Institute, Parkville, Australia,* [2]*Ministry of Health and Medical Services, Honiara, Solomon Islands,* [3]*Australian National University, Canberra, Australia,* [4]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [5]*St Vincent's Hospital, University of New South Wales, Sydney, Australia,* [6]*Kirby Institute, University of New South Wales, Sydney, Australia*

Scabies is caused by infestation with a microscopic mite and can lead to impetigo from secondary bacterial infection and further serious complications. Many Pacific Island Countries, including Solomon Islands, have a high burden of scabies and accompanying bacterial skin infection. This study was conducted as part of the Regimens of Ivermectin for Scabies Elimination (RISE) study. RISE is a cluster randomised non-inferiority trial of one versus two doses of ivermectin mass drug administration (MDA) in Western Province in Solomon Islands. Prior to MDA skin examinations were conducted in all participants to determine baseline prevalence of scabies and impetigo. Twenty remote villages scattered across ten islands participated in the study. Each village had a population between 200 and 500 people and all residents were invited to participate. Diagnosis of scabies was made using the International Alliance for the Control of Scabies criteria. Nurses conducted skin examination of exposed body areas and interviewed participants for history features. We conducted skin examinations on 5239 participants, representing over 95% of the total eligible population. Overall scabies prevalence was 16.1% (95% CI 12.5-20.7), ranging from 3.3% to 46.8% by village. There was a higher prevalence of scabies in males (17.8%, adjusted risk ratio 1.2 compared to females). Scabies prevalence was highest in infants (27.8%, adjusted risk ratio 2.9 compared to 50-59 year olds). Overall impetigo prevalence was 5.6% (95% CI 4.2-7.3), ranging from 1.4% to 19.0% by village. Males had a greater burden of disease (6.5%, adjusted risk ratio 1.4). Impetigo prevalence was highest in two to four year olds (11.1%, adjusted risk ratio 9.5 compared to 50-59 year olds). Participants with scabies had a higher prevalence of impetigo (10.3% compared to those without scabies 4.6%, risk ratio 2.2, 95% CI 1.8-2.7). There is a high burden of scabies in this population of the Solomon Islands. Scabies and impetigo are a considerable public health challenge in Solomon Islands and understanding the burden of disease is an important step towards development of control strategies, including MDA.

81

SURVEILLANCE OF TICK-BORNE INFECTIONS IN LIVESTOCK IN THE GUINEA SAVANNA AREA OF NORTHERN GHANA

Seth Offei Addo[1], Victor Asoala[2], Charlotte Addae[1], Mosore Mba-tihssommah[1], Bernice A. Baako[2], Stephen Kantum[2], Abraham Oduro[2], Deborah Narwortey[2], Michael Wiley[3], Randal J. Schoepp[4], Jeffery W. Koehler[4], Keersten Ricks[4], Sean Griffin[5], Suzanne Mate[6], John A. Larbi[7], Philip K. Baidoo[7], Michael D. Wilson[1], Shirley Nimo-Paintsil[8], James F. Harwood[9], David Wolfe[8], Andrew G. Letizia[10], Joseph W. Diclaro II[11], Yvonne-Marie Linton[12], Samuel K. Dadzie[1]

[1]*Noguchi Memorial Institute for Medical Research, Accra, Ghana,* [2]*Navrongo Health Research Centre, Navrongo, Ghana,* [3]*University of Nebraska Medical Center, Omaha, NE, United States,* [4]*U.S Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, United States,* [5]*Engineering Research Development Center-Geospatial Research Lab, Fort Belvoir, VA, United States,* [6]*U.S. Army Emerging Infectious Diseases Branch, Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [7]*Department of Theoretical and Applied Biology, College of Science, Kumasi, Ghana,* [8]*U.S Naval Medical Research Unit No. 3, Ghana Detachment, Accra, Ghana,* [9]*U.S Naval Medical Research Unit No. 3, Sigonella, Italy,* [10]*Infectious Diseases Department, Naval Medical Research Center, Silver Spring, MD, United States,* [11]*Navy Entomology Center for Excellence, Jacksonville, FL, United States,* [12]*Walter Reed Biosystematics Unit, Smithsonian Institute, Suitland, MD, United States*

Ticks are the second most important vector, transmit diverse pathogens that affect both animal and human health. With the global threat of emerging or re-emerging tick-borne diseases, it is essential to conduct frequent surveillance to inform on the risks of zoonotic transmission of pathogens. In most parts of Ghana where livestock are kept as a source of income, nutrition, and social capital, there is a significant risk of zoonotic infections. This study was conducted in Navrongo, located in the Guinea Savanna zone of Ghana, very close to Burkina Faso where animal husbandry and farming are the main occupations of the inhabitants. Preliminary sampling of ticks from cattle revealed the primary tick species (>90%) to be *Amblyomma variegatum.* Initial results indicated that the ticks were infected with zoonotic pathogens: *Rickettsia africae* (65.9%), undescribed *Babesia* species (86.4%), *Ehrlichia ruminantium* (4.5%), and other uncharacterized *Ehrlichia* (2.3%) species. Co-infections were found in 59% of ticks with pathogens Babesia/*Rickettsia africae* (52.3%),*Babesia*/uncharacterized *Ehrlichia* species/*Rickettsia africae* (2.3%), Babesia/*Ehrlichia ruminantium*/*Rickettsia africae* (2.3%) and *Babesia*/*Ehrlichia ruminantium* (2.3%). Subsequent, bloodspot samples and additional ticks have been collected from cattle, chicken, sheep and goats pending molecular and serological testing. Initial findings from this study indicate that ticks in Navrongo are infected with *Rickettsia africae* and have a high occurrence of *Babesia* species. The public health implications of these findings in the study area reveal pathogen threats to the farming and livestock community, and surrounding communities which pose risks to occupational and environmental exposure to illness with no current treatment or preventive measures.

82

AN EPIDEMIOLOGICAL SEARCH FOR THE ASIAN LONGHORNED TICK IN SOUTH CAROLINA

McKenzi Norris, Kyndall Dye-Braumuller, Danielle Johnson, Connor Ross, Chloe Rodriguez Ramos, Melissa Nolan

University of South Carolina, Columbia, SC, United States

In 2017, *Haemaphysalis longicornis*, the Asian longhorned tick, was first reported in the United States. Within months, nine states reported *H. longicornis*, highlighting its success as an invasive species domestically. To explore the exposure potential posed by *H. longicornis* on cattle and humans in neighboring South Carolina, ticks were collected from state parks and from cattle ranches. Bimonthly collections took place at state parks and private ranches throughout the Upstate, Pee Dee, Midlands, and Lowcountry regions of South Carolina. At each site, ticks were collected

by performing tick dragging and using CO_2-baited traps. A second independent method of sampling specifically targeting *H. longicornis* involved grooming cows at partnered livestock ranches in the four regions of South Carolina. The cattle were sampled once a month at each ranch during the months of June and July (what we anticipated as peak activity months for the adult ticks). The results of our study are advising veterinary and public health officials in South Carolina and surrounding states about the potential presence of *H. longicornis*.

83

AEDES SPECIES BREEDING: IMPORTANCE OF CONTAINER SIZE AND WATER REFILLING FREQUENCY IN MOMBASA COUNTY, KENYA

Gathenji Njoroge[1], Francis Mutuku[2], Jenna Forsyth[3], Mwashee Lutt[4], Amy Krystosik[5], Eleanor Frost[6], A. Desiree LaBeaud[7]

[1]*UC Berkeley School of Public Health, Berkeley, CA, United States,* [2]*Technical University of Mombasa, Environment and Health Sciences Department, Mombasa, Kenya,* [3]*Stanford University, Stanford Woods Institute for the Environment, Stanford, CA, United States,* [4]*Vector-borne Disease Control Unit, Msambweni, Kenya,* [5]*Stanford University, School of Medicine, Department of Pediatrics, Division of Infectious Disease, Stanford, CA, United States,* [6]*Stanford School of Medicine, Stanford, CA, United States,* [7]*Stanford University, School of Medicine, Stanford, CA, United States*

Numerous studies have documented the relationship between uncovered containers and vector-borne diseases. *Aedes* spp. mosquitoes (vectors of the dengue and chikungunya viruses) can reproduce in water collected in uncovered or discarded containers, creating the potential for disease outbreaks to occur, particularly in low-income or urban areas. To address this problem, we conducted an education-based intervention to empower elementary-age children in Mombasa County, Kenya to decrease the prevalence of uncovered containers around their houses. The data analyzed in this paper were collected at baseline and at three months post-intervention to assess whether machine learning models could be trained to predict the presence of immature mosquitoes in water samples and identify which model performs best. The Area Under Receiver Operating Characteristic (AUROC) metric was used to assess model performance, as it accounts for the large imbalance in the dataset. There were 530 households enrolled in the study: 271 in the intervention and 259 in the control arm. Containers (e.g. plastic drums, small domestic containers, tires) outside each house were examined for the presence of immature mosquitoes and labeled by house and container for analysis. Among 29 other features, the number of immature mosquitoes in each positive container was recorded. There were 110 positive containers and 1897 negative containers. The best performing model was the Gradient Boosting Classifier, with an AUROC score of 0.898. The three features with the greatest odds ratios were: Container was Filled Last Week (OR = 35.14), Container was Filled Last Month (OR = 35.11), and Container is Large (OR = 11.22). Optimizing for AUROC, the Gradient Boosting Classifier screens for the predicted presence of immature mosquitoes in a given water container at a study house. This could increase the efficiency of vector management initiatives. Large containers and containers filled with water last week or last month were associated with the presence of immature mosquitoes and should be targeted in community education and vector management initiatives.

84

SEROPREVALENCE OF ANTIBODIES AGAINST *TRYPANOSOMA CRUZI* AND ARBOVIRUSES IN DOMESTIC DOGS IN TWO ZOOGEOGRAPHICAL REGIONS OF MEXICO

Edward Davila[1], Héctor Ochoa Díaz-López[2], Nadia A. Fernández-Santos[3], Mario A. Rodríguez-Perez[3], Jose Guillermo Estrada-Franco[3], Susana Arellano Chávez[2], Lihua Wei[3], Jesús A. Aguilar-Durán[3], Matthias Frank[4], Monica Borucki[4], Estelle Martin[1], Alex Pauvolid-Correa[1], Sarah Hamer[1], Gabriel Hamer[1]

[1]*Texas A&M University, College Station, TX, United States,* [2]*El Colegio de La Frontera (ECOSUR), San Cristobal de Las Casas, Mexico,* [3]*Instituto Politécnico Nacional, Centro de Biotecnología Genómica, Reynosa, Mexico,* [4]*Lawrence Livermore National Laboratory, Livermore, CA, United States*

Vector-borne diseases continue to emerge worldwide in human and animal populations. In the Americas, major pathogens of concern include the mosquito-borne arboviruses (e.g., chikungunya, Zika and dengue viruses) and *Trypanosoma cruzi,* the protozoan parasite spread by triatomine insects that causes Chagas disease. Our overall aim is to determine the degree to which domestic dogs may serve as sentinels for these infections. Specific objectives include (i)determine evidence of prior exposure to arboviruses based on the presence of neutralizing antibodies; and (ii) determine the prevalence of canine infection with different strains *T. cruzi*. Using a cross-sectional study design, dog blood samples were collected from two endemic regions: Reynosa, Tamaulipas (northern Mexico) and Tuxtla Gutierrez, Chiapas (southern Mexico). Whole blood and plasma were tested for antibodies to *T. cruzi* using the STAT-PAK rapid immunochromatographic assay and Indirect Fluorescent Antibody (IFA). DNA was extracted from whole blood and tested for *T. cruzi* DNA using qPCR. For positive samples, amplification and sequencing of the spliced leader intergenic region (SL-IR) was used to determine parasite strain type. Sera are being tested for neutralizing antibodies to Zika and other arboviruses using plaque reduction neutralization tests (PRNT) to determine monotypic reactions. Overall, seroprevalence of dogs to *T. cruzi* was higher in northern Mexico (27%; n=183) than southern Mexico (18%; n=113; P=0.0705) using STAT-PAKS and two of these samples were also positive on IFA. Of 231 dogs tested for *T. cruzi* DNA, 8 were positive (3.4%), although the SLIR region failed to amplify for 4 samples, likely due to low parasite load. The other 4 have yet to strain be strain-typed. Of 294 dogs screened for neutralizing antibodies to Zika via PRNT, 2 were potentially seropositive. Of 281 dogs screened for DENV-2, 8 were potentially seropositive for neutralizing antibodies. Given the widespread distribution of dogs and their close associations with humans, characterizing the epidemiology of canine vector-borne disease may provide data to help predict and manage human disease.

85

DOGS AND TICKS AS EPIDEMIOLOGICAL SENTINELS: SURVEILLANCE OF TICK-BORNE HUMAN PATHOGENS IN CIUDAD JUAREZ (CHIHUAHUA), NEAR THE MEXICO-U.S. BORDER

Diana Marcela Beristain-Ruiz[1], Javier A. Garza-Hernandez[1], Susana Ordonez-Lopez[1], Julio V. Figueroa-Millan[2], S. Viridiana Laredo-Tiscareno[3], Jose J. Lira-Amaya[2], Andres Quezada-Casasola[1], Beatriz Alvarado-Robles[1], Oliver Castillo-Luna[1], Adriana Floriano-Lopez[1], Carlos A. Rodriguez-Alarcon[1]

[1]*Universidad Autonoma de Ciudad Juarez, Juarez, Mexico,* [2]*CENID Salud Animal e Inocuidad. Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias., Jiutepec, Mexico,* [3]*Department of Veterinary Microbiology and Preventive Medicine, Ames, IA, United States*

Tick-Borne Bacterial Diseases (TBBDs) are known to cause Ehrlichiosis, Borrelioses, Anaplasmosis, and Rickettsiosis that have great impacts on public health. Epidemiological studies using domestic animals as sentinels are used for surveillance of infectious diseases, including the TBBDs. That methods can provide essential surveillance information useful to identify dynamics in the infection and/or health status of animal and

human populations, to know patterns of pathogen diversity, to controlling and preventing diseases timely in a relatively inexpensive manner, etc. Thus, dogs can play useful role as sentinel hosts for monitoring diseases. The dogs live in close communion with humans and livestock and are susceptible to many emerging or re-emerging human vector-borne infections as the TBBDs. By other hand, the ticks can be used to monitoring TBBDs; the use of hematophagous arthropods to survey vertebrates for the presence of infectious disease agents are called xenosurveillance and it has been very well documented. The aim of this study was to identify the tick species that naturally parasitize the dogs that live in Cd. Juárez, as well as to identify the presence of pathogens that can transmit to their hosts. 432 dogs were sampled and of which 1691 ticks were obtained; the 99.82 % of the ticks collected in this study was morphological identify as *R. sanguineus s.l.,* while the remaining 0.18% were identified *as O. megnini*. Only 194 dogs were sampled, and PCR analysis were made in order to identify tick-borne pathogens. Infection of *E. canis* were detected in 53.60% of dogs, *A. platys* in 24.74%, *A. phagocytophilum* in 12.88% and *R. rickettsi* in 5.67 %. Coinfections of these pathogens were also found in 21.6% of dogs. *B. burgdorferi s.l.* was not detected. To date, there are no formal studies of ticks and tick-borne diseases in this geographic area, but the possibility that brown dog ticks could transmit diseases to humans justifies prevalence studies; although the dog is its primary host, this tick can parasitize other animals, including humans.

86

DNA BARCODING REVEALS NEW RECORDS OF POTENTIAL ZOONOTIC NEARCTIC BLACK FLIES (DIPTERA: SIMULIIDAE) FROM CHIHUAHUA STATE, NORTHWEST MEXICO

Javier Alfonso A. Garza-Hernandez[1], Catherine C. Martínez-Hernández[1], Stephanie V. Laredo-Tiscareño[2], Carlos A. Rodríguez-Alarcón[1], Diana M. Beristain-Ruíz[1], Jaime R. Adame Gallegos[3], Rodolfo González- Peña[3], Luis M. Hernández-Triana[4]

[1]*Universidad Autónoma de Ciudad Juárez, Ciudad Juárez, Chihuahua, Mexico,* [2]*Iowa State University, Ames, IA, United States,* [3]*Universidad Autónoma de Chihuahua, Chihuahua, Chihuahua, Mexico,* [4]*Animal and Plant Health Agency, Addlestone, United Kingdom*

The state of Chihuahua covers a surface area of 247,455 square kilometers, making it the largest state in Mexico. There are no systematic and updated studies on blackfly fauna, only 10 species have been registered. To document the diversity and distribution of black flies inhabiting the state, collection trips were conducted in the subregion of Tarahumara Mountain ranges and Plains belonging to the physiographic region of Sierra Madre Occidental. Thus, blackflies specimens (larvae, pupae, reared adults) were collected in the period 2018 in and near rivers and streams. No invasive Hotshot technique was used for DNA extraction. COI mitochondrial region was sequenced from the specimens collected. In total, we collected 138 specimens representing 7 species. Of these, all of them are new records for Chihuahua state: *Simulium ochraceum, S. samboni, S. quadrivittatum, S. paynei, S. pulverulentum, S. virgatum, and S. marquezi*; DNA barcode sequences of these species were recovered. The overall genetic distance in the dataset (n=95) was 0.13%. In this study, we assessed the use of the cox1 DNA barcoding region for the identification of species of blackflies in Chihuahua, Mexico. Our results showed that using the DNA barcoding with elementary knowledge on morphometrical taxonomic, it is possible to identify the blackflies species in Mexico. Also, these results indicate a new distribution of Neotropical blackflies along the Nearctic region that are incriminated as one of the main vectors of onchocerciasis in Latin America.

87

EVALUATING THE COMPETENCY OF THE INVASIVE MOSQUITO SPECIES, *AEDES J JAPONICUS*, IN TRANSMITTING VARIOUS JAPANESE ENCEPHALITIS VIRUS GENOTYPES

Astri N. Faizah[1], Daisuke Kobayashi[2], Haruhiko Isawa[2], Michael Amoa-Bosompem[3], Kozue Miura[1], Kazuhiro Hirayama[1], Kyoko Sawabe[2]

[1]*The University of Tokyo, Tokyo, Japan,* [2]*National Institute of Infectious Diseases, Tokyo, Japan,* [3]*Tokyo Medical and Dental University, Tokyo, Japan*

Japanese encephalitis virus (JEV) is a mosquito-borne flavivirus that is currently the leading cause of viral encephalitis in Asia. The virus is likely maintained in an enzootic cycle between swine, waterbirds, and *Culex* mosquitoes. For almost a century, it has been circulated indigenously in Asia, with *Culex tritaeniorhynchus* as the primary vector.In some areas where the primary vector is practically absent, sporadic cases of JE have been reported and *Aedes j. japonicus* presumed to be the secondary vector. As one of the world's most expansive culicid species, it carries a considerable health risk that it may spread diseases to wider areas. From its original distribution range in East Asia, there are reports of previously established populations across continents including Europe and North America. Thus evaluation of their competency as JE vector, particularly from a native strain, is dearly required to prevent potential disease spread in the future.In this study, the mosquitoes' vector competence was assessed, through exposure to various JEV genotypes (i.e. genotype I-V) with *Cx. tritaeniorhynchus* as positive control. Virus-containing blood was membrane-fed to the mosquitoes and infection, dissemination, and transmission rates evaluated by either RT-qPCR or viral propagation in a mammalian cell line.Preliminary results from two dominant genotypes (GI and GIII) showed an infection and dissemination rate of 10-12% in *Aedes j. japonicus*, indicating successful viral propagation in the mosquito midgut and viral spread into tissues and body parts. Although the primary vector, *Cx. tritaeniorhynchus*, exhibited 80-90% of infection and dissemination rate, the fact that JEV was established in *Aedes j. japonicus* is of public health significance.Furthermore, with a transmission rate of six percent, they can transmit the JEV to the next host. This may explain the human cases and infrequent detection in primary vector-free areas in Japan. Importantly, *Aedes j. japonicus*, could be a relevant vector spreading the disease into new areas, thus security measures need to be taken in areas where the mosquito is distributed, or where it may be introduced.

88

HABITAT PRODUCTIVITY OF MALARIA VECTORS IN AREAS WITH INTEGRATED ADULT VECTOR CONTROL IN WESTERN KENYA; POTENTIAL FOR TARGETED LARVAL CONTROL

Pauline W. Orondo[1], Harrysone Atieli[2], Steven G. Nyanjom[1], MingChieh Lee[3], Andrew K. Githeko[4], Guiyun Yan[5]

[1]*Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya,* [2]*International Center of Excellence for Malaria Research, Kenya, Homabay, Kenya,* [3]*University of California, Irvin, CA, United States,* [4]*Kenya Medical Research Institute, Kisumu, Kisumu, Kenya,* [5]*University of California, Irvin, USA, Irvin, CA, United States*

Anopheles arabiensis and *An. funestus* are the major malaria vectors in the semi-arid southern low lands of the Lake Victoria basin where malaria transmission was perennial and hyper-endemic prior to LLINs and IRS interventions. *An. funestus,* a highly anthropophilic and endophagic vector population radically declined leaving significant populations of largely zoophilic but potentially anthropophilic *An. arabiensis*. This study was done to determine breeding sites of surviving *An. funestus* and *An. arabiensis* populations. This in turn could lead to effective application of larval control using short-term Bti/Bs and slow release long-term formulations. The study was done in Homa Bay, an area with concrete-based irrigation canals, streams and rivers. Six replicates of different habitat types (rice paddies, fish ponds, man- made ponds, drainage ditches and swamps) were selected and 2.5 m^2 emergence traps used to capture emerging adult vectors every night. Larval sampling and adult aspiration

were done every morning. Adult males were discarded. The traps were relocated every 2 days. In each habitat 180 trap collections were carried out. *Culex* larval densities were high in all habitat types (65.9-117.1 larvae/ dip). The larval densities of *An. arabiensis* and *An. funestus* were inversely related to instar age indicating high larval mortality or predation. The highest mean densities of *An. arabiensis* were collected in man- made ponds (14.1 larvae/ dip) and lowest in swamps (5 larvae/ dip). The mean densities of emerged female *An. arabiensis* collected in rice paddies were 0.04/ night. *An. funestus* was only found in rice paddies. Adult *Culex* was in abundant in swamps (2/ trap). Other anophelines collected included *An. coustani* and *An. pharoensis* with the highest mean densities trapped in drainage ditches (0.17/ trap) and rice paddies (0.12/ trap) respectively. These results indicate that although immature stages are abundant in the aquatic habitats, few develop into adult vectors. This study also suggests that inasmuch as there are reduced adult vectors in circulation, further reduction can be achieved by applying targeted larval source management.

89

DESCRIPTION OF BLOOD MEAL SOURCES FOR DIFFERENT *ANOPHELES* SPECIES IN MALARIA ENDEMIC AREAS OF HONDURAS

Denis Gustavo Escobar Robles, Krisnaya Ascencio, Andres Ortiz, Adalid Palma, Gustavo Fontecha

Microbiology Research Institute - Universidad Nacional Autonoma de Honduras, Tegucigalpa, Honduras

Malaria transmission is highly influenced by behavior of *Anopheles* mosquitoes. The blood feeding patterns and host preferences of anophelines species have not been adequately studied in Mesoamerica. In the current context of malaria elimination, it is recommended to update the knowledge of the bionomics of vectors in order to improve vector control tools. Mosquito collections were carried out at eight malaria endemic sites in Honduras between February and October 2019. A DNA-based approach was used to determine five potential blood meal sources, including human, bovine, dog, chicken and pig. Also, to determine mosquito infections by malaria parasite, PCR was made on individual mosquitoes from departments of Gracias a Dios and El Paraíso. Our analysis included 311 anophelines; 181 (58%) mosquitoes of seven *Anopheles* species were visually engorged: *An. albimanus* (n=107), *An. darlingi* (n=20), *An. crucians* (n=20), *An. neivai* (n=5), *An. pseudopunctipennis* (n=4), *An. punctimacula* (n=2) and *An. vestitipennis* (n=23). The most frequent blood meal sources were chicken (29.5%) and bovine (27.5%). Regarding human blood detection, 40 anophelines from 4 species were positive, with an average human blood index (HBI) of 22.1%. *An. darlingi* showed the highest HBI with 55% followed by *An. albimanus* (25%). Finally, 29.8% of blood sources belonged to unidentified origins, especially in Gracias a Dios. Analysis of mixed blood meals shows that most mosquitoes fed on more than one host (n=82). 24.9% and 27.6% fed on one and two different sources, respectively. *Plasmodium* spp infected mosquitoes could not be detected in this study. Our results showed multiple origins of blood meal for most anophelines, however, there is still an important proportion of unidentifiable feeding sources. *Anopheles* species in Honduras have shown high plasticity in the selection of hosts to feed, especially in Gracias a Dios. In conclusion, our results suggest that animals around households are important food sources for anophelines, and contribute to maintaining vector populations in malaria endemic areas. This poses major challenges for vector control of malaria in Mesoamerica.

90

EXPANSION OF THE DISTRIBUTION OF *AEDES ALBOPICTUS* IN ARMENIA 2016-2019

Lusine Paronyan[1], Gegham Pashinyan[1], Lilit Babayan[1], Arsen Manucharyan1[1], Haykuhi Vardanyan[1], Ruben Danielyan[1], Francis Schaffner[2], Vincent Vincent Robert[3]

[1]*National Center for Disease Control and Prevention, MoH, Yerevan, Armenia,* [2]*Francis Schaffner Consultancy, Riehen, Swaziland,* [3]*National Institute of Research for Sustainable Development, MIVEGEC Unit, Montpellier Univ., IRD, CNRS, Montpellier, France*

Aedes albopictus and *Ae. aegypti* mosquitoes have been found in the territories bordering Armenia. A VectorNet field mission of Armenia in 2016 identified 29 different species of mosquitoes, including Invasive species Ae. albopictus for the first time, that was recorded in a single locality, at the border point with Georgia, on the main road Tbilisi-Yerevan. Data of routine entomological surveillance (conducted by field entomologists in NCDC branches) in 2017-2019 were used for presence/ absence of Aedes invasive species, obtained at a selection of sample sites, and gathered during the mosquito active season (April-November). Classical entomological methods were used, immature aquatic stages by dipping and adults were collected with CO_2 baited traps. Human landing catches were performed in the daytime. Sample identification was performed for larval and adult stages based on standard morphological keys with external quality control by NCDC reference laboratory. Only adults of Aedes albopictus were recorded in 2017 in the same locality as in 2016, border point Bagratashen, 450m above sea level. In 2018 entomological investigation recorded its establishment (adults and larvae) and spread in northern Armenia up to 15 km in Ayrum town (500 MAMSL). In 2019 adults and larvae of Aedes albopictus were recorded 60km inland from border point in Ijevan town, again on the main road Tbilisi-Yerevan (750 MAMSL). Aedes albopictus, an important potential vector of many arboviruses, was recorded during the four consecutive years 2016-2019. Field observations demonstrate its recent introduction and establishment in the north of the country, with implications for public health. More comprehensive studies are required to understand the real distribution of Aedes albopictus in Armenia, to estimates and predict the future distributions of it in response to target surveillance and control efforts that aim to mitigate the spread of arboviral diseases among the population.

91

LONGEVITY OF *AEDES AEGYPTI* IN OPEN-AIR INSECTARIES IN KISUMU, WESTERN KENYA

Bryson A. Ndenga[1], Joel O. Mbakaya[1], Vincent O. Were[1], Uriel Kitron[2], Francis M. Mutuku[3], A. Desiree LaBeaud[4]

[1]*Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya,* [2]*Department of Environmental Sciences, Emory University, Atlanta, GA, United States,* [3]*Department of Environment and Health Sciences, Technical University of Mombasa, Mombasa, Kenya,* [4]*Department of Pediatrics, Stanford University, Stanford, CA, United States*

The question of how long *Aedes aegypti* mosquitoes live remains poorly studied. This experimental study estimated the longevity of *A. aegypti* mosquitoes in open-air insectaries. Ovitraps were set to collect *A. aegypti* eggs, larvae were reared on plastic trays, adults were reared in netted cages, fed on sugar solution and females by membrane feeding on fresh cow blood. Durations in each of their developmental stages were recorded daily until they all died. A total of 1,844 eggs were collected and 1,148 (62.3%) hatched. Eggs hatched within seven days; most hatched on the second day. Temporal overlaps were observed in all the six *A. aegypti* developmental stages from first instar larvae to adults. Males emerged and died earlier than females. On average, *A. aegypti* took three to four days to develop through each aquatic stage, six days as adults prior to blood-feeding, 10 and 14 days for adult males and females, respectively, after providing the first blood-meal. On average, counting from hatching day, *A.*

aegypti adult males and females lived for 34 and 37 days, respectively. The longevity of *A. aegypti* adult females, in this area, is sufficient to sustain the incubation and transmission of viruses like dengue and chikungunya.

92

EDUCATIONAL INTERVENTION IMPROVES SOURCE REDUCTION KNOWLEDGE AND ATTITUDES IN URBAN KENYA

Eleanor Frost[1], Jenna Forsyth[1], Arielle Kempinsky[1], Gathenji Njoroge[1], Mwashee Lutt[2], Francis Mutuku[2], Desiree LaBeaud[1]

[1]*Stanford University, Stanford, CA, United States,* [2]*Technical University of Mombasa, Mombasa, Kenya*

Aedes spp. mosquitoes spread diseases that can be curtailed by source reduction to decrease mosquito breeding. Our educational intervention sought to improve knowledge, attitudes, and self-reported behaviors regarding mosquito bite prevention in adults living in Likoni in Mombasa county, Kenya, Africa. 530 heads of households were enrolled with 271 assigned to intervention and 259 to the control arm. We implemented a cluster randomized controlled trial in coastal Kenya, assigning five communities to an educational intervention and five to control. We report on the three-month follow up from September 2019 pertaining to changes in self-reported knowledge, attitudes, and behaviors that contribute to vector breeding. We determined the reliability of self-reported outcomes using Kappa analyses to understand the significance and veracity of our results. Knowledge and behaviors cumulative scores reflected their responses to multiple questions in either category. The attitudes score was treated as a binary outcome based off the distribution. The intervention group demonstrated an improvement in self-reported knowledge, attitudes, and behaviors. On average, the intervention parents scored 1.05 points higher on their knowledge cumulative score and 1.8 points higher on their behaviors cumulative score than control participants ($p<.0001$ and $p<.0001$). Intervention participants had a 20% increased risk in answering all attitude questions correctly compared to the control ($p=.0002$). Participants responded to similar questions consistently (Kappa coefficient of .55 (.452-.641)). This educational intervention improved mosquito prevention knowledge leading to reported source reduction behavior changes in the short-term. Poisson and linear regressions and Kappa analyses indicate that participants' knowledge, attitudes, and behaviors improved at three months post-intervention. At one-year post-intervention (Summer 2020), these same three outcomes will be analyzed to determine sustainability of source reduction attitudes and behaviors due to this intervention.

93

ENDOPHAGIC AND EXOPHAGIC BEHAVIOR IN *ANOPHELES COLUZZII* AND *ANOPHELES GAMBIAE* CARRYING THE *L1014F KDR* MUTATION IN BENIN

Germain Gil Padonou[1], Albert Salako[1], Rock Aikpon[1], Razaki Ossé[1], Fortuné Dagnon[2], Daniel Impoinvil[3], Seth Irish[3], André Sominahouin[1], Aurore Ogouyèmi Hounto[4], Martin Akogbéto[1]

[1]*Centre de Recherche Entomologique de Cotonou, Ministère de la Santé, Cotonou, Benin,* [2]*US President's Malaria Initiative US Agency of International Development, Cotonou, Benin,* [3]*US President's Malaria Initiative, Centers for Disease Control and Prevention, Atlanta, GA, USA 30329, GA, United States,* [4]*Programme National de Lutte contre le Paludisme, Ministère de la Santé, Cotonou, Benin*

This study investigated the relationship between knock-down resistance (kdr) genotypes and *Anopheles gambiae* s.l. feeding frequency indoors (endophagy) and outdoors (exophagy) in Benin. In 2018, *Anopheles* mosquitoes were collected indoors and outdoors by human landing catches in Benin departments of Alibori, Donga and Ouémé, during the dry and rainy season. Molecular analyses were used to identify the species of the *An. gambiae* complex and detect the presence of kdr mutations. Crosstabs/Chi-square tests were used to compare the frequency of the kdr alleles of each species and the indoor/outdoor feeding frequency. A total of 1078 *An. gambiae* s.l. specimens were analyzed from the departments. The only kdr resistance allele detected was L1014F. The frequency of the kdr L1014F allele was higher in *An. gambiae* than in *An. coluzzii* in Alibori ($p<0.001$), while it was similar between *An. gambiae* and *An. coluzzii* in Donga ($p=0.189$) and Ouémé ($p=1.000$). Homozygous-resistant (*L1014F/L1014F*) individuals of *An. gambiae* were more endophagic than exophagic in Alibori ($p=0.020$), Donga ($p=0.001$) and Ouémé ($p<0.001$), whereas heterozygous-resistant (*L1014F/L1014L*) and wild-type non-resistant (*L1014L/L1014L*) individuals had similar biting frequency indoors and outdoors in the three study areas ($p=1.000$). In contrast, *An. coluzzii* had similar indoor and outdoor biting frequency for homozygous ($p=0.259$) and heterozygous, ($p=0.071$) and wild type ($p=0.290$) kdr genotypes in the Alibori and Donga. In Ouémé, homozygous ($p<0.001$) and heterozygous ($p=0.002$) kdr genotypes of *An. coluzzii* were more exophagic than endophagic, while the wild-type genotype was more endophagic ($p<0.001$). These results suggest variability in the relationship between kdr and biting behavior among sibling species of the *An. gambiae* s.l. Different genotypes seemed to influence endophagy and exophagy of *An. gambiae* and *An. coluzzii* in different ways. This relationship may also be influenced by spatial and temporal factors. Further studies are needed to elucidate if and how resistance mechanisms shape malaria vector biting behavior and impact vector control interventions.

94

IMPACT OF RECENT WEATHER EXTREMES ON MOSQUITO-BORNE DISEASE TRANSMISSION IN KENYA

Cameron Nosrat[1], Jonathan Altamirano[2], Assaf Anyamba[3], Jamie M. Caldwell[1], Richard Damoah[3], Francis Mutuku[4], Bryson Ndenga[5], Desiree LaBeaud[2]

[1]*Stanford University, Stanford, CA, United States,* [2]*Stanford University School of Medicine, Stanford, CA, United States,* [3]*NASA Goddard Space Flight Center, Greenbelt, MD, United States,* [4]*Technical University of Mombasa, Mombasa, Kenya,* [5]*Kenya Medical Research Institute, Nairobi, Kenya*

Climate change and variability influence temperature and rainfall, impacting vector abundance and thus the dynamics of vector-borne disease transmission. The fingerprint of climate change is observed in warming ocean temperatures, which are projected to increase the frequency and intensity of extreme weather events. Mosquito-borne diseases, such as dengue fever, are primarily transmitted by *Aedes aegypti* mosquitoes. Globally, dengue is the fastest spreading vector-borne disease. Water availability and local temperatures affect populations of dengue vectors via reproduction and thus the ability to effectively transmit the disease; however, the effect of droughts, floods, heat waves, and cold waves is not well understood. Using vector, climate, and disease data collected between 2013-2019 in Kenya, this study aims to answer how periods of extreme rainfall and temperature affect mosquito abundance and the risk of arboviral infections. In order to define periods of variability in rainfall and temperature, we calculated monthly anomalies of the variables with respect to their long-term means (1983-2019, 2000-2019) across four study locations in Kenya. Anomalies were defined as the upper and lower 10% of these LST or rainfall differences. Monthly *Ae. aegypti* abundance was assessed by collaborators in Kenya using four trapping methods. Blood samples were collected from children with acute febrile illness presenting to field sites and tested for dengue virus using IgG-ELISA and PCR. We found that mosquito eggs were significantly more abundant one month following an abnormally wet month and that adult mosquitoes were more abundant one month following an abnormally cool month. Such increases in mosquito abundance did not contribute to increases in confirmed dengue cases 2 months following the anomaly, as would be expected by the cycle of infection. While our findings do not present a significant increase in dengue cases following periods of extreme weather, an increase in mosquitoes suggests increased risk for arboviral infections. Targeted interventions after extreme weather events are necessary to reduce risk of viral transmission.

95

ENTOMOLOGICAL SURVEILLANCE OF MALARIA VECTORS IN FIVE SENTINEL SITES IN CAMEROON

Etienne Fondjo[1], Etienne E. Fondjo[1], Jean-Claude J. Toto[2], Magellan M. Tchouaki[3], Ekoko E. Ekoko Wolfang[2], Salemon S. Patchoke[4], Benjamin B. Menze[3], Boris B. Njeambosay[5], Raymond R. Tabue[4], Elysee E. Mandeng[2], Emmanuel E. Elanga[3], Elysee Choutpouen[2], Edmond E. Kopya[2], Dorothy D. Achu[4], Kelley K. Ambrose[6], Judith J. Hedje[7], Celestin C. Kouambeng[7], Jenney J. Carlson[8], Sarah S. Zohdy[9], Joseph J. Chabi[10]

[1]*Vectorlink Cameroon, Yaounde, Cameroon,* [2]*Central African Organization for Endemic Disease Control, Yaounde, Cameroon,* [3]*Centre for Research in Infectious Diseases, Yaounde, Cameroon,* [4]*National Malaria Control Programme, Yaounde, Cameroon,* [5]*Biotechnology Center, Yaounde, Cameroon,* [6]*Abt associates, Rockville, MD, United States,* [7]*Agency for International Development, Yaounde, Cameroon,* [8]*Agency for International Development, USA, WA, United States,* [9]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [10]*Vectorlink Cameroon, Abidjan, Côte D'Ivoire*

From October 2018 to September 2019, the U.S. President's Malaria Initiative VectorLink Cameroon Project conducted malaria vector monitoring in five sentinel sites—monthly in Simatou and Gounougou in the North and every two months in Mangoum, Nyabessang, and Bonabéri in the South. Human landing catches (HLCs), pyrethrum spray catches, and U.S. Centers for Disease Prevention and Control light traps were used to collect adult mosquitoes in households to assess vector composition, human biting rate (HBR), endophagic index, indoor resting density, parity and infection rates, and entomological inoculation rate (EIR). In January 2019, the end time of HLCs was extended from 6:00 am to 8:00 am to assess variability in vector biting time. *Anopheles* species collected across sites included *An. gambiae* s.s., *An. coluzzii*, *An. arabiensis*, *An. funestus* s.s., *An. leesoni*, *An. nili*, *An. moucheti*, *An. demeilloni*, *An. pharoensis*, *An. ziemanni*, *An. multicinctus*, and *An. marshallii*, all of which are confirmed malaria vectors in the country. Hybrids of *An. gambiae*/*An. coluzzii* (0.5%) were found in Simatou, Mangoum, and Nyabessang and *An. funestus* s.s. (74.7%) and *An. leesoni* (25.3%) were found in Simatou and Gounougou. The mean HBR of *Anopheles* indoors ranged from 12 bites/person/night (b/p/n) in Bonabéri to 94 b/p/n in Simatou, and outdoors from 21 b/p/n in Mangoum to 91 b/p/n in Simatou. *An. gambiae* s.l. and *An. moucheti* in Mangoum, Bonabéri, and Nyabessang continued to bite until 8:00 am. The average *Anopheles* indoor resting density was 19.4 females/room/night and the average parity rate was 68.9%. High monthly EIRs were recorded across sites, with 41.5 infective bites/person/month (ib/p/m) in Gounougou, 80.5 ib/p/m in Simatou, 53.6 ib/p/m in Mangoum, 34.8 ib/p/m in Nyabessang, and 27.6 ib/p/m in Bonabéri. The trends may be due to few vector control activities, including low ITN coverage, in the country. The results, particularly the high EIRs, highlight the urgent need for integrated vector control interventions to reduce malaria transmission and burden in Cameroon, and can help inform National Malaria Control Program decisions on selection and deployment.

96

CHARACTERIZING EARLY-FORAGING ANOPHELINES IN A HOLOENDEMIC MALARIA SETTING IN NCHELENGE DISTRICT, ZAMBIA

Mary E. Gebhardt[1], David Mbewe[2], Francis Kapasa Mulenga[2], James Sichivula[2], Mbanga Muleba[2], William J. Moss[1], Jennifer C. Stevenson[3], Douglas E. Norris[1]

[1]*Johns Hopkins Bloomburg School of Public Health, Baltimore, MD, United States,* [2]*Tropical Disease Research Centre, Ndola, Zambia,* [3]*Macha Research Trust, Macha, Zambia*

Malaria is one of the leading causes of preventable death and is transmitted by *Anopheles* mosquitoes commonly recognized to bite indoors and late at night. This behavior allows for tools like insecticide-treated bed nets (ITNs) and indoor residual spraying (IRS) to effectively prevent malaria transmission in many malaria-endemic settings. In Nchelenge District, Zambia, malaria is transmitted year-round, with 50-80% of individuals parasitemic at any given time despite a decade of intensive annual IRS and ITN distributions. The limited effectiveness of these generally proficient interventions has cast doubt on our understanding of the canonical feeding behaviors of the major vector, *Anopheles funestus*, and led to a concerning hypothesis that this vector may be feeding outdoors and before people go indoors to sleep. We performed entomological collections over eight weeks in Nchelenge District by setting CDC light traps indoors, outdoors where people gather, and near animal pens from 16:00-22:00, before people go under their bed nets to sleep. In addition, we assessed human risk by performing surveys to collect information about evening behaviors, and collected environmental and household data to identify other covariates associated with high proportions of early-biting mosquitoes. At least nine species of *Anopheles* were identified that foraged outdoors and indoors before people go under their bed nets in the evening, including a large proportion of *Anopheles gibbinsi* - a species which was not previously described in Zambia. We aim to use epidemiological and environmental data to identify behavioral, ecological, and household-level risk factors associated with these early-biting anophelines. These data will help inform recommendations to improve malaria control strategies in this region of Zambia where malaria remains holoendemic and to provide a potential explanation for the refractoriness to traditional vector control interventions.

97

ENTOMOLOGICAL INDICATORS OF MALARIA TRANSMISSION IN SIERRA LEONE: IMPLICATIONS FOR VECTOR CONTROL

Evelyne Alyko[1], Samuel Juana Smith[2], Yemane Yihdego[3], Rebecca Levine[4], Jenny Carlson[5], Tony Hughes[4], David Schnabel[6], Ramlat Jose[7], Stephen Mansaray[1], Miriam Mokuena[3], Frederick Yamba[2]

[1]*Abt Associates Inc, Freetown, Sierra Leone,* [2]*National Malaria Control Program, Freetown, Sierra Leone,* [3]*Abt Associates Inc, Rockville, MD, United States,* [4]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [5]*US Agency for International Development, Washington, DC, United States,* [6]*President's Malaria Initiative, Centers for Disease Control and Prevention, Freetown, Sierra Leone,* [7]*President's Malaria Initiative, US Agency for International Development, Freetown, Sierra Leone*

We assessed entomological indicators for malaria vectors in Sierra Leone by collecting mosquitoes monthly from houses in rural and peri-urban sites between May 2018 and February 2019, from the four regions of the country. *Anopheles* females were morphologically classified, then analyzed molecularly for species identification, malaria parasite detection, and host blood-meal origin. Composition of the *Anopheles* species was: 98.5% *An. gambiae* s.l., 1% *An. funestus* s.l., 0.4% *An. coustani*, and 0.04% *An. ziemanni*. *An. gambiae* s.l. were further classified as 74.7% *An. gambiae* and 25.3% *An. coluzzii*. The overall mean density of *An. gambiae* s.l. per house per day was 12.8, with 14.5 in rural sites and 11 in peri-urban sites. Significantly more ($p < 0.001$) *An. gambiae* s.l. were collected indoors (52%) compared to outdoors (48%). The highest overall biting rate occurred in June, with a higher frequency indoors (73.4 bites/person/night - b/p/n) compared to outdoors (60.5 b/p/n). Overall indoor biting activity peaked after 10 PM, and most human-vector contact occurred after 12:00 AM. The entomological inoculation rate (EIR) was highest in July overall (40.5 infective bites/person/month - i/b/p/m), as well as for peri-urban sites both indoors (36.5 i/b/p/m) and outdoors (46.7 i/b/p/m), and in rural sites outdoors (58.7 i/b/p/m); the EIR was highest in August in rural sites indoors (62.4 i/b/p/m). The proportion of blood-fed *An. gambiae* s.l. caught indoors was high at both rural (53.8%) and peri-urban (46.7%) sites, with a human blood index of 88% and 75%, respectively. Since malaria vector control in Sierra Leone relies primarily on insecticide treated nets (ITNs), high coverage and use of ITNs should have an impact on malaria due to vectors' preference for feeding indoors, with most human-vector contact occurring late at night. If indoor residual spraying is

used for vector control, implementation should occur in April, before the wet season begins and mosquito population densities and biting rates, as well as EIR, reach their peak. These findings underscore the importance of using entomological indicators for vector control decision-making.

98

THE DAILY COLLECTION OF MOSQUITO SALIVA ON BLOTTING PAPER PADS ALLOWS FOR THE LONGITUDINAL MONITORING OF VIRAL TRANSMISSION FROM *CULEX TARSALIS* MOSQUITOES EXPOSED TO WEST NILE VIRUS

Jeremy P. Ledermann, Paul L. Burns, Lara Perinet, Ann M. Powers, Nathaniel M. Byers

Centers for Disease Control and Prevention, Fort Collins, CO, United States

Laboratory vector competence studies are typically performed to determine the potential for a particular species of mosquito to transmit a given arbovirus. We have developed and characterized a 12-well plate mosquito housing containment system and saliva collection method that is a safer, more efficient, and sensitive procedure when compared to the traditional glass capillary method. This new system was utilized to monitor the ability of *Culex tarsalis* (KNWR) adult mosquitoes to transmit virus after exposure to West Nile virus (WNV). Paper filter pad samples from individual mosquitoes were collected from two cohorts starting on day 3 post exposure (DPE) through either 8 DPE (n=26) or 12 DPE (n=23). Saliva samples eluted from these pads were assayed for viral nucleic acid by real-time RT-PCR. The earliest detection of viral RNA was observed on 4 DPE with 8.2% (4/49) of the pads positive with a maximum transmission rate of 69.6% (16/23) on 9 DPE. When aggregated data was calculated for longitudinal samples, the rate increased to 43.4% (10/23) and 84.6% (22/26) for the 8 and 12 DPE cohorts, respectively. In addition, this data shows that 32 mosquitoes were WNV RNA positive on at least 1 of the 10 collection days, 20 were positive for multiple collection days, and 14 were positive on 3 or more consecutive days. The ability to monitor arboviral vector competence in individual mosquitoes over time increases the likelihood of the detection of positive saliva and allows for the collection of longitudinal data to elucidate critical components of the infection process in mosquitoes.

99

ENTOMOLOGICAL CHARACTERIZATION OF *AEDES* MOSQUITOES AND ARBOVIRUS DETECTION IN IBAGUÉ, COLOMBIA

María C. Carrasquilla[1], Mario I. Ortiz[1], Cielo M. León[1], Silvia Y. Rondón[1], Beate Sander[2], Benoit Talbot[3], Manisha Kulkarni[3], Heriberto Vásquez[4], Camila González[1]

[1]*Centro de Investigaciones en Microbiología y Parasitología Tropical CIMPAT, Universidad de los Andes, Bogotá, Colombia,* [2]*Toronto General Hospital Research Institute, University Health Network; Institute of Health Policy, Management and Evaluation, University of Toronto; Public Health Ontario; ICES, Toronto, ON, Canada,* [3]*School of Epidemiology and Public Health, University of Ottawa, Ottawa, ON, Canada,* [4]*Secretaría de Salud de Ibagué, Ibagué, Colombia*

In Colombia, dengue is an endemic disease that shows peaks of transmission; meanwhile chikungunya and Zika have been recently introduced, generating epidemics during the periods 2014-2015 and 2015-2016 respectively. These diseases are caused by arboviruses that are transmitted by the mosquitoes *Aedes aegypti* and *Ae. albopictus*. The objective of this study was to perform an entomological characterization of *Aedes* vectors, and to detect the circulation of arbovirus in Ibagué, a city that has endemoepidemic patterns of dengue transmission and that reported a high prevalence of chikungunya and Zika during the epidemic periods. Sampling was performed monthly between June 2018 and May 2019 in neighborhoods of different socioeconomic status. Mosquitoes were collected using BG sentinel, CDC and resting traps, as well as Prokopack aspirators. In mosquitoes, molecular detection of dengue, Zika and chikungunya viruses was performed using Multiplex Real-Time RT-PCR. *Aedes aegypti* was collected in all sampled neighborhoods and *Ae. albopictus* was reported for the first time in Ibagué. In total, 1463 *Ae. aegypti* and 7 *Ae. albopictus* mosquitoes were collected. Females were more abundant than males (804 vs 659, respectively; $p<0.05$). A greater abundance of mosquitoes were collected in low compared to high socioeconomic status neighborhoods (950 vs 513, respectively; $p<0.05$) and more mosquitoes were captured, using the aspirator, indoors than outdoors (1004 vs 195, respectively; $p<0.05$). Females that tested positive for virus were collected in low socioeconomic status neighborhoods. In total, four *Ae. aegypti* pools (0.888%) were positive for dengue virus serotype 1 (DEN-1) and one for chikungunya virus (0.222%). In conclusion, dengue and chikungunya viruses were detected in *Ae. aegypti* collected in Ibagué. These infected mosquitoes were only collected in low socioeconomic status neighborhoods. Zika virus was not detected in mosquitoes, three years after the introduction and the epidemic period of this virus in the country. A higher abundance of mosquitoes indoors than outdoors, increases the risk of arbovirus transmission.

101

IMPACT OF THE SOUTHERN OSCILLATION INDEX, TEMPERATURE, AND PRECIPITATION ON EASTERN EQUINE ENCEPHALITIS VIRUS ACTIVITY IN FLORIDA

Kristi M. Miley, Joni A. Downs, Sean P. Beeman, Thomas R. Unnasch

University of South Florida, Tampa, FL, United States

Eastern Equine Encephalitis virus (EEEV) is the most pathogenic mosquito borne illness affecting the eastern United States, especially Florida. Effects of the Southern Oscillation Index (SOI), precipitation, and cooling degree days on EEEV horse case data in Florida during 2004 to 2018 were modeled using distributed lag non-linear models (DLNMs). The analysis was conducted at two spatial scales: statewide and regional. DLNMs were used in order to model the potential delayed effects of the covariates on monthly counts of horse cases. Both spatial scale models confirmed a seasonal trend in EEEV transmission and found that precipitation, cooling degree days, and the SOI were all predictors of monthly numbers of horse cases ($p < 0.05$). EEEV activity in horses was associated with: (1) higher amounts of rainfall during the month of transmission at the statewide scale, as well as the prior three months at the regional scale, (2) fewer cooling degree days during the month of transmission and the preceding three months, and (3) high SOI values during the month and the previous two months, as well as low SOI values in the prior 2 to 8 months. Horse cases were lower during El Niño winters but higher during the following summer, while La Niña winters were associated with higher numbers of horse cases, with fewer cases during the following summer. At the regional scale, extremely low levels of precipitation were associated with a suppression of EEEV cases for three months. Given the periodicity and potential predictability of ENSO cycles, precipitation, and temperature, these results may provide a method for predicting EEEV risk potential in Florida that may be utilized in prevention and control strategies.

102

ESTIMATED ARTHRALGIA RESOLUTION RATE IN CHRONIC CHIKUNGUNYA DISEASE: A META-ANALYSIS OF LONGITUDINAL COHORT STUDIES

Hugh Robert Watson[1], Aileen Y. Chang[2], Giulia Calusi[3], Denise Federico[3], Marco Pergher[3]

[1]*Evotec ID (Lyon), Lyon, France,* [2]*George Washington University, Washington, DC, United States,* [3]*Aptuit Srl, Verona, Italy*

Observational studies have reported widely varying frequency of chronic arthralgia at different time-points after chikungunya infection. There is a consensus that most arthralgia resolves over time, but the rate of resolution of symptoms is uncertain. Our objective is to estimate the average expected rate of resolution of chronic post-chikungunya arthralgia from cohorts with repeated follow-up over time. Meta-analysis was performed on published reports of chronic chikungunya disease cohorts.

Criteria for inclusion were: enrolment of patients at the acute phase of disease, clinical follow-up at more than one time-point ≥3 months after onset of acute symptoms, follow-up of >80% of enrolled cases. Monthly declines in symptom prevalence were calculated for each interval between reported follow-up times for each cohort. Mean monthly resolution rates were derived for reported arthralgia prevalence, and ranges calculated by considering missing cases to be either all symptomatic or all non-symptomatic. Eight cohorts reporting arthralgia prevalence at multiple follow-up times were identified. Cohort sizes ranged from 50 to 509 with a total of 1,825 patients. One cohort originated from Europe, 2 from Asia/Indian Ocean and 5 from the Americas/Caribbean. Follow-ups spanned the interval of 3 to 40 months. Resolution rate curves were obtained suggesting that 23% (range 22-23%) of patients symptomatic at 3 months will have resolved by 6 months, 49% (range 44-50%) by 12 months and 71% (range 63-79%) of patients by 24 months. Predicted arthralgia prevalence exhibited a one-phase exponential decline over the first 40 months from infection. We estimated from intra-cohort changes that the mean rate of symptom resolution shows an exponential decay leading to the probability that a residual small proportion of patients will have persisting symptoms long-term.

104

FINE-SCALE MAPPING OF MAYARO VIRUS OCCURRENCE IN LATIN AMERICA REVEALS TRANSMISSION HOTSPOTS IN BRAZIL & PERU

Michael J. Celone[1], Barbara Han[2], Simon Pollett[3]

[1]*Uniformed Services University of the Health Sciences, Bethesda, MD, United States,* [2]*Cary Institute of Ecosystem Studies, Millbrook, NY, United States,* [3]*Viral Diseases Branch, Walter Reed Army Institute of Research, Silver Spring, MD, United States*

Mayaro Virus (MAYV) is a zoonotic vector-borne *Alphavirus* that has caused outbreaks of febrile illness in Latin America. Although the Pan American Health Organization has emphasized the need for increased awareness of this emerging virus, the precise areas of spillover risk from MAYV remain unclear. Granular maps of MAYV risk are therefore critical to guide surveillance and control efforts. We curated a comprehensive geodatabase and fine-scale map of human MAYV infections in the Americas. We searched the PubMed, Embase, ProMed, GIDEON, and SCIELO databases through January 2020, collectively. Grey literature review included recent systematic reviews of MAYV epidemiology and pre-print servers. All confirmed human cases of MAYV infection were geocoded by decimal degrees (DD) latitude and longitude geographic coordinates in ArcGIS 2.4.0. For cases reported by administrative level only, we used the centroid of the finest level. One-hundred thirty-eight unique locations of human MAYV infections were recorded between 1954 - 2020, including Antigua, Bolivia, Brazil, Colombia, Costa Rica, Ecuador, French Guiana, Guatemala, Guyana, Haiti, Mexico, Panama, Peru, Suriname, Trinidad and Tobago, and Venezuela. MAYV was reported most frequently in Para, Brazil (22 reported occurrences) followed by Goiás, Brazil (17 reported occurrences), Mato Grosso, Brazil (16 reported occurrences) and Loreto, Peru (15 reported occurrences). Moran's Global I test revealed statistically significant clustering of MAYV cases and spatial structure of this emerging virus [Moran's Index = 0.06, $p < 0.001$]. In addition, time-stratified MAYV occurrence mapping indicated increased MAYV detection in the last 20 years compared to historical data, especially in Brazil, Ecuador and Peru. Furthermore, 13 tourists were diagnosed with MAYV in Europe or North America after traveling to Latin America. The tourists had visited Peru (n=4), Bolivia (n=2), Brazil (n=2), French Guiana (n=2), Suriname (n=2), and Ecuador (n=1). Further study is essential to identify specific demographic and ecological risk factors for MAYV spillover in these and other countries in the Americas.

105

SILENT REEMERGENCE OF CHIKUNGUNYA IN THE NORTHERN COAST RURAL COMMUNITIES OF ESMERALDAS

Sully Marquez[1], Gabriel Trueba[1], Joseph N. Eisenberg[2], Josefina Coloma[3]

[1]*Universidad San Francisco de Quito, Quito, Ecuador,* [2]*University of Michigan, Michigan, MI, United States,* [3]*University of California, Berkeley, CA, United States*

Chikungunya (CHIKV) is a vector borne virus that belongs to the genus *Alphavirus*, family Togaviridae, with a positive single stranded RNA genome. The main vectors for this virus are the mosquitoes *Aedes aegypti* and *Aedes albopictus*. In 2013, the CHIKV Asian genotype emerged in the Americas and spread rapidly through Caribe, Central and South America. From 2014 to 2018, Ecuador reported 35,722 cases of CHIKV, from these, 10,791 cases were reported in Esmeraldas province. Interestingly in 2019, only dengue cases (n=1,750) and no Chikungunya cases were officially reported in this province. Reverse transcriptase-PCR performed in 160 febrile serum samples collected from May to October 2019 in 6 rural communities revealed that 23 samples were positive for dengue, 110 samples were positive for Chikungunya, and 14 corresponded to coinfections of CHIKV and dengue virus. Our data indicated that Chikungunya and dengue were co-circulating and were responsible for most febrile cases in this region in 2019. These communities are located near the Colombian border, a country which reported 238 cases of CHIKV in 2019.

106

POLYCLONAL ANTIBODIES PREVENT EFFECTS OF CHIKUNGUNYA VIRUS INFECTION IN MICE

Aruni Jha[1], Douglas Barker[1], Xiaobing Han[1], Hongyu Qiu[1], Eryu Wang[2], Scott Weaver[2], Justin Julander[3], Cory Nykiforuk[1], Shantha Kodihalli[1]

[1]*Emergent BioSolutions, Winnipeg, MB, Canada,* [2]*University of Texas Medical Branch, Galveston, TX, United States,* [3]*Institute of Antiviral Research, Utah State University, Logan, UT, United States*

Chikungunya virus (CHIKV) causes a mosquito-borne disease endemic to parts of sub-Saharan Africa, Southeast Asia, tropical areas of the Indian subcontinent and islands in the south-western Indian Ocean, and Latin America. The infection causes debilitating disease, with long-term consequences including arthralgia-like symptoms. There are no approved drugs or vaccines to treat CHIKV infections. A chikungunya equine polyclonal Fab/F(ab)2 antibody product (CHIKV-EIG) is being developed for the acute treatment of CHIKV infections. This report describes the efficacy of CHIKV-EIG against acute CHIKV infection in immunocompetent and immunocompromised mice. Immunocompetent mice treated prophylactically with CHIKV-EIG showed significantly better outcomes than vehicle controls after infection with the wild-type CHIKV-LR strain, including complete prevention of footpad swelling at the site of infection, weight loss and virus burden. Treatment of immuno-compromised (IFNAR1 -/-) mice with CHIKV-EIG after infection resulted in significantly enhanced survival, reduction in virus burden and reduced severity of clinical signs of infection compared to controls. These data show that the CHIKV-EIG reduces mortality and morbidity of CHIKV infection in immuno-compromised and immunocompetent mouse models of acute disease.

107

EXPECTED ENDPOINTS FROM FUTURE CHIKUNGUNYA VACCINE TRIAL SITES INFORMED BY SEROLOGICAL DATA AND MODELING

Quan Minh Tran[1], James Soda[1], Amir Siraj[1], Sean Moore[1], Hannah E. Clapham[2], Alex Perkins[1]

[1]*University of Notre Dame, South Bend, IN, United States,* [2]*National University of Singapore, Singapore, Singapore*

The large-scale expansion of chikungunya virus over the last several years has resulted in increased interest in developing a vaccine for this disease. However, planning for a chikungunya vaccine trial is challenging due to its sporadic and unpredictable transmission. The trial should be responsive and carefully planned. This motivates us to come up with a new methodology to quantify the number of endpoints, which is one of an important quantity when planning a vaccine trial. We present a new approach for informing the number of end-point events for vaccine trials that is applicable to chikungunya. We first accounted for population immunity using serological data that were collated from a systematic review and were then used to estimate the basic reproduction number (R_0) of previous outbreaks, given models with different numbers of historical outbreaks. Then, the infection attack rate (IAR) of a future epidemic was projected based on the estimated R_0 and population immunity at the time of a future trial. Estimates of IAR were then used to inform how many endpoints could be expected if trials were to take place in these sites and an outbreak were to occur. For most sites, statistical support was strongest for models that assumed that there have been either one or two outbreaks in the population historically. We also observed that sites for which R_0 was estimated to be higher and population immunity lower were projected to experience a larger number of endpoints in the event of a future outbreak. The approach that we present does not depend on real-time incidence data like traditional methods but does require serological data, which are likely to be measured for trial planning anyway. Validating these projections of epidemic size against future epidemics will be important for refining this method and making it a more reliable basis for trial planning. This framework could also be extended to other pathogens.

108

CLINICAL LESSONS FROM DETAILED EVALUATION OF A CHRONIC CHIKUNGUNYA COHORT IN GUADELOUPE

Remi Bossy[1], Hugh Watson[2], Denise Federico[3], Anne-Laurence Demoux[4], Nadia Rugard[5], Giulia Calusi[3], Julien Dezaunay[6], Franciane Gane-Troplent[1], **Fabrice Simon**[7]

[1]*Université des Antilles, Pointe-à-Pitre, Guadeloupe,* [2]*Evotec ID, Virology, Lyon, France,* [3]*Biometry, Aptuit Srl., Verona, Italy,* [4]*CHU Nord, Marseille, France,* [5]*Cabinet médical, Le Gosier, Guadeloupe,* [6]*CREPS, Les abymes, Guadeloupe,* [7]*Unité des Virus Emergents, Marseille, France*

A cohort of patients with chronic chikungunya musculoskeletal symptoms in Guadeloupe were evaluated in order to determine the most relevant and practical clinical screening tools for identifying severe chronic chikungunya. Sixty-one patients (51 female and 10 male), followed up a mean of 36 months after chikungunya infection, underwent detailed clinical examination for musculoskeletal involvement, assessment of subjective symptoms, and the impact on mood, physical activity and quality of life (SF12). RAPID3 was used as an inflammatory arthritis global score. Spearman correlations and principal component analysis were performed. Patients had extensive musculoskeletal involvement shown by a mean RAPID3 score of 16.95 (out of 30), and by 9±4 tender joints, stiffness in 5±4 joints and synovitis in 2±3 joints, most frequently in the distal joints of the lower and upper limbs, and in the lumbar region. Impact on global disease scores (RAPID3) and quality of life was not obviously driven by any particular musculoskeletal segments, although upper arm involvement was most closely correlated. Simple patient scores of pain, stiffness impact and asthenia appeared to be good indicators of overall disease severity and poor quality of life. The emergence of anxiodepressive syndromes were also associated with poor quality of life and interruption of work, and with the number of musculoskeletal segments involved. These data suggest that patient assessment of pain, stiffness and asthenia may be useful and simple tools to identify the patients most in need of specialist referral, and that anxiodepressive syndromes should be recognised for their important association with chronic chikungunya disease.

109

FUNCTIONAL CHARACTERIZATION OF MV-CHIK INDUCED ANTIBODY RESPONSES

Rukie De Alwis[1], **Roland Tschismarov**[2], Raphael Zellweger[3], Leong Yan Shan[1], Erich Tauber[2], Katrin Ramsauer[2]

[1]*Duke-NUS Medical School, Singapore, Singapore,* [2]*Themis Bioscience, Vienna, Austria,* [3]*International Vaccine Institute, Seoul, Republic of Korea*

MV-CHIK, a measles-vectored vaccine for the prevention of Chikungunya disease, has demonstrated a highly favorable safety and tolerability profile in multiple phase 1 and 2 clinical trials. Further, it was repeatedly shown that the vaccine candidate induces substantial amounts of neutralizing antibodies, representing a likely correlate of protection, in trial participants receiving one or two administrations. To better characterize qualitative aspects of the humoral response elicited in humans upon vaccination, sera from a subset of participants of a phase 2 trial were used for a series of experiments. We previously reported that IgG subtyping revealed an antibody response dominated by IgG1 and IgG3, with slightly higher avidity achieved after two vaccinations. Here we show that MV-CHIK induced antibodies additionally facilitate antibody-dependent cellular phagocytosis as well as antibody-dependent cytotoxicity, thereby replicating key aspects of protective humoral responses mounted after natural infection.

110

CHIKUNGUNYA INFECTION IN HUMAN NEURONS AND CEREBRAL ORGANOIDS

Kelli Barr, Emily Schultz, Tyanthony Jones, Bernd Zechmann, Dana Dean

Baylor University, Waco, TX, United States

Chikungunya virus (CHIKV) recently emerged in the Western Hemisphere where outbreak reports from this region have documented unprecedented levels of neurological disease, congenital infections, and death. Factors contributing to arboviral neuro-pathogenesis have been studied in animals, but this research hasn't translated to human systems. As a result, we still don't understand the mechanisms that cause arboviral induced neurological disease. In order to bridge this gap, we investigated the effects of CHIKV infection in human cerebral organoids as well as dopaminergic and GABA neurons derived from human induced pluripotent stem cells. We infected GABA and DOPA neurons and mature cerebral organoids with CHIKV and collected data on size, viral kinetics, and gene expression. We also obtained images to visualize where CHIKV localized in neural tissue and to observe physiological changes in the organoids over the course of 14 days. We performed immunofluorescence for markers associated with neurotransmission, innate immunity, and neurodegeneration to validate gene expression data. Both organoids and neurons exhibited dysregulation of genes linked with mood and mental disorders.

112

GENOME SEQUENCES OF CHIKUNGUNYA VIRUS ISOLATES FROM BOLIVIA

Caio Martinelle B. França[1], Roxana Loayza[2], Yelin Roca[2], Ana Maria Montaño Arias[2], Freddy Tinajeros[3], Jose R. Loiza[4], Anshule Takyar[5], Robert H. Gilman[6], Matthew J. Miller[7]

[1]*Southern Nazarene University, Bethany, OK, United States,* [2]*Centro Nacional de Enfermedades Tropicales (CENETROP), Santa Cruz, Plurinational State of Bolivia,* [3]*Asociación Benéfica PRISMA, Santa Cruz, Plurinational State of Bolivia,* [4]*Instituto de Investigaciones Científicas y*

Servicios de Alta Tecnología (INDICASAT AIP), City of Knowledge, Panama, [5]Department of Public Health and Department of Microbiology, University of Oklahoma, Norman, OK, United States, [6]Bloomberg School of Public Health, John Hopkins University, Baltimore, MD, United States, [7]Sam Noble Museum of Natural History, University of Oklahoma, Norman, OK, United States

In Bolivia, chikungunya virus was first detected in early 2015, with cases of disease peaking between March and May. Here, we report nine chikungunya (*Togaviridae: Alphavirus*) genome sequences of isolates from Bolivia. Febrile patients were screened for chikungunya virus at the Cenetrop national tropical medicine laboratory. We selected nine archived samples (maximum of 1 passage) for sequencing. All isolates came from blood-extracted RNA positive by qPCR. Seven of the nine samples were from Santa Cruz de la Sierra, one sample from Cochabamba and one sample from Trinidad. We generated cDNA using random hexamers via RT-PCR. We amplified the chikungunya genome using a multiplex tiled amplicon approach. All samples were pooled and sequenced on a single Oxford Nanopore MinION R9.4 flow cell, generating 2,776,384 reads. Base-calling was done in real time using Albacore v2.3.1. We demultiplexed and trimmed adapters and barcodes using qcat v1.1.0 The average read length for QC-passed reads was 325.4 bp (range, 100 to 3,727 bp). For our highest read count sample (4866-15), we error corrected, trimmed, and de novo assembled reads in Canu v1.9. The resulting assembly was fragmented, so we selected the largest contig to identify the closest whole chikungunya genome on GenBank using BLAST to guide reference-based assembly. We mapped these reads to the sequence of KY703969.1 using Minimap2 implemented in Geneious v2020.0.5. The final sequence length for all nine genomes is 11,182 nucleotides representing 99.6% of the nonstructural and structural coding regions. We generated a maximum likelihood phylogeny using IQ-Tree v2.0-rc1. We found that the nine Bolivian sequences are part of the widespread Asian-Caribbean chikungunya genotype and form a unique clade that was part of a larger monophyletic lineage primarily containing sequences from Nicaragua, Aruba, Colombia, and the United States. The monophyly of our nine samples supports the hypothesis that a single lineage was widely circulating in Bolivia during the early 2015 chikungunya outbreak.

114

DENGUE: RECOVERY VACCINES

Scott B. Halstead[1], Timothy Endy[2]

[1]*Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [2]New York Upstate Medical Center, Syracuse, NY, United States*

Dengvaxia, a chimeric yellow fever tetravalent dengue vaccine developed by SanofiPasteur has been licensed in several dengue-endemic countries. Dengvaxia protected children who were partially immune to dengue virus (DENV) but sensitized seronegative children to breakthrough disease of enhanced severity. In 2019, based upon WHO recommendations, the European Medicines Agency and the US FDA issued licenses that reconciled safety issues by restricting vaccine to individuals who had one or more prior DENV infections. Dengvaxia has been licensed in 20 dengue endemic countries without such restriction. In the Philippines in 2016, over 800,000 9 year-old children were given Dengaxia. Using Sanofi serostatus efficacy and published seroepidemiological data for that country it can be predicted that during a period of 4 years post-vaccination there will be more than one thousand seronegative children hospitalized for vaccine-related severe dengue and, in addition, large numbers of severe cases among vaccinated seropositives. Enhanced surveillance should be provided to these at-risk children to enable quick access to medical care for those who develop vaccine enhanced disease. Vaccinated seronegative children acquire a serostatus equivalent to that of monotypic DENV-immunes, thus they are at risk to antibody enhanced infections (ADE) for the rest of their lives. Can they be rescued? Lifelong immunity follows sequential infection with two different DENV. This event can be mimicked using recovery vaccines: 1) one dose of any of the attenuated DENV 1, 2 or 4 developed by Mahidol University and licensed to Aventis or 2) a single dose of the Takeda live-attenuated vaccine, TAK 003. Scientific and ethical issues will be discussed.

115

DENGUE VACCINATION COVERAGE IN A SUBNATIONAL VACCINATION CAMPAIGN IN THE STATE OF PARANÁ, BRAZIL

Karin Regina Luhm[1], Clara Preto[1], Angela Maron de Mello[2], Eliane Malluf[1], Gabriel Graeff[3], Gabriela Sousa[1], Lineu Silva[1], Magda Clara Vieira da Costa Ribeiro[1], Marilene Buffon[1], Sílvia Shimakura[1], Sônia Raboni[1], Denise Siqueira de Carvalho[1]

[1]*UFPR, Curitiba, Brazil, [2]FUNASA, Curitiba, Brazil, [3]FUNPAR, Curitiba, Brazil*

According to recent estimates, 390 million dengue infections occur annually and 128 countries are at risk of infection. In Brazil, more than 1.6 million dengue infections were reported in 2015 only. The strategy used to prevent transmission, through vector control, has proved to be expensive and not very effective. The first dengue vaccine, Dengvaxia®, was licensed in Brazil in 2015. This study aimed to describe dengue vaccination coverage (VC) in a subnational vaccination campaign, that took place in 30 municipalities in Paraná state, Brazil. A descriptive cross-sectional study was carried out based on dengue vaccination campaign data, performed between August 2016 and December 2018. The vaccine was provided freely for people aged 15 to 27 years old in 28 municipalities, and aged 9 to 44 years in two municipalities, representing a target population of 500,000 people. Vaccinated individuals were categorized according to the number of received doses, considering sex, age group and municipality. VC, vaccination dropout rate (DR) and compliance rate with vaccine schedule (CR) were calculated and 95% confidence intervals (95% CI) were estimated. During the campaign, 302,603 individuals received at least one dose of the vaccine in the 30 municipalities. The total VC for at least one dose of vaccine was 60.5%, 44.2% for at least two and 28.6% for three. The DR was 52.8%. Among individuals vaccinated with three doses, CR was 87.0%. Overall, smaller municipalities had higher VC compared to larger ones. A higher percentage of men started vaccination (males 64.0%, 95% CI 63.8-64.1%; females 57.1%, 95% CI 57.0-57.3%), however, there was a higher DR among men (males 55.4%, 95% CI 55.2-55.7%; females 49.9%, 95% CI, 49.6-50.1). The VC were significantly lower in individuals aged 20 to 27 either for at least one dose (9-19 67.4%, 20-27 51.9%, 28-44 89.3%) or for three doses (9-19 35.5%, 20-27 21.1%, 28-44 48.8%). The results highlight the challenges in reaching appropriate VC, especially as regards to the completion of the vaccination schedule. The differences in VC between age, sex and municipalities size show the need for customized vaccination strategies.

116

DENGUE FEVER PREVENTION AND WEATHER FACTORS

Leonor Castro Loffredo[1], Rodolpho Telarolli Junior[1], Bruno Lian Segantini[2], Christian Wagner Maurencio[2], Fabiano Santos Galego[2], João Ramalho Borges[2], Társis Eschaquetti Benevides[2]

[1]*UNESP - Universidade Estadual Paulista and UNIARA/Universidade de Araraquara, Araraquara, Brazil, [2]UNIARA - Universidade de Araraquara, Araraquara, Brazil*

Since the first epidemics in the 1950s, dengue fever has been neglected by the national public policies, despite being a serious public health problem. Dengue affects more than a hundred countries worldwide. In Brazil, more than 2 million cases have been reported, and neigher the state of São Paulo, the richest in the Federation, has been spared. And even, its Central region, the most prosperous in which the municipality of Araraquara is located, has suffered from epidemics of the disease. Araraquara has a population of nearly 230 thousand inhabitants and reported, in 2019, 25,973 dengue cases. In previous years, dengue fever increased year by year, as a result of climatic factors favorable to the vector's proliferation, in addition to the absence of robust and permanente public policies aimed at eliminating *Aedes aegypti* breeding sites. Among the multiple factors related to the occurrence of dengue fever, we had

studied the monthly incidence of the disease in Araraquara, from 2012 to 2016, and its correlation with the environmental temperature and rainfall rates. The annual incidence coefficients (per 100,000 inhabitants) were 52.68, 376.52, 737.39, 3,660 and 809.48 respectively to 2012, 2013, 2014, 2015 and 2016. It was observed significant correlation coefficients between the number of dengue cases and climatic variables. Temperature values of a specific month presented significant correlation with the number of dengue cases after 2 months (r= 0.7972), 3 months (r=0.9231), and 4 months (r= 0.8322). On the other hand, pluviosity rates of a specific month presented significant correlation with the number of dengue cases after 1 month (r=0.5944), 2 months (r= 0.8881), 3 months (r=0.9231) and 4 months (r=0.7063). Higher temperature and rainfall create a scenario with more favorable conditions for emergence of dengue fever and other diseases borne by *Aedes aegypti* mosquitoes. These findings, associated with robust health education measures and the appropppriate health surveillance actions, help the public authorities to prepare and rationalize the allocation of resources throughout the year to combat the dengue vector mosquito.

117

A NOVEL MULTI-LEVEL COMMUNITY-BASED ACTIVE SURVEILLANCE SYSTEM TO DETECT DENGUE IN NORTHERN ECUADOR ALONG A RURAL-URBAN GRADIENT

Jessica Uruchima[1], Sully Márquez[2], Amanda VanEngen[1], William Cevallos[3], Gwenyth O. Lee[1], Gabriel Trueba[2], Josefina Coloma[4], Joseph N. Eisenberg[1]

[1]*University of Michigan, Ann Arbor, MI, United States,* [2]*Universidad San Francisco de Quito, Quito, Ecuador,* [3]*Universidad Central del Ecuador, Quito, Ecuador,* [4]*University of California, Berkeley, CA, United States*

Dengue national surveillance programs provide important national scale information. However, passive surveillance's low sensitivity and high rate of misclassification is insufficient to identify local dengue transmission for targeted intervention. To address the need for more sensitive dengue case detection over a large region, we employed a novel community-based active surveillance in an epidemiological study of arbovirus transmission. Reported health symptoms from 6 communities (n = 4981) along a rural-urban gradient in Esmeraldas province, Ecuador were screened to identify arbovirus-like febrile cases, which were tested for dengue virus. Adapted from a community-based approach used in Nicaragua, our surveillance used a multi-level triage approach. The first level of triage comprised non-medically trained community members called brigadistas. Through weekly (high season) or biweekly (low season) visits to neighbors, brigadistas actively captured health symptoms occurring within the last 7 days of their visit. Reported fever, rash or red eyes triggered a referral to a study auxiliary nurse. At the second level of triage, auxiliaries used an in-depth questionnaire to isolate dengue-like febrile cases from among referrals. Blood samples from these febrile cases underwent a third level of laboratory analyses. From May 2019 to December 2019, brigadistas visited 4294 individuals at least once, representing 86% of the censused population. Brigadistas referred 439 symptomatic individuals to auxiliaries, 31% (n = 135) of which were labeled as arbovirus-like disease. Laboratory analyses confirmed 35% (n = 47) of these arbovirus-like symptoms as dengue cases. During the same period, the Ministry of Health identified 38 dengue cases in the same communities, with lower case detection in the more remote rural communities. Inclusion of non-medically trained local field workers made surveilling multiple communities possible and produced data complementary to that of the national surveillance system. In the future, the brigadista system may be useful in promoting community-based intervention and research strategies.

118

LOW-COST, COMMUNITY-DRIVEN VECTOR-CONTROL FOR DENGUE SUPPRESSION IN THE GREATER MEKONG SUB-REGION

Leo Braack[1], Thy Chea[2], Srun Set[2], Sokha Suan[2], Mark Debackere[2], Pierre Echaubard[3], Jeffrey Hii[4], Bernadette Ramirez[5], Jacob Bigio[6], John Hustedt[2], Hans J. Overgaard[7]

[1]*Malaria Consortium, Bangkok, Thailand,* [2]*Malaria Consortium, Phnom Penh, Cambodia,* [3]*University of London, London, United Kingdom,* [4]*James Cook University, Cairns, Australia,* [5]*World Health Organization, Geneva, Switzerland,* [6]*McGill University Health Centre, Montreal, QC, Canada,* [7]*Norwegian University of Life Sciences, Oslo, Norway*

A widely quoted 2013 publication estimated the annual number of global dengue cases at about 390 million, while more recent findings indicate a trend of doubling in case numbers every 10 years. Dengue control is a challenge because of a lack of effective dengue vaccines and treatments. Therefore, locally-adapted vector control augmented by effective communication is currently the mainstay for controlling the disease, thereby decreasing the incidence of infection and preventing disease outbreaks. The mosquitoes responsible for dengue transmission are *Aedes aegypti* and *Aedes albopictus*, the same vectors responsible for transmission of chikungunya, Zika, and other diseases. These mosquitoes breed in household water-storage containers, tires, solid waste and other water-holding containers and receptacles. We carried out a cluster randomized controlled trial in Cambodia using community mobilization for deployment of more than 25,000 larvivorous fish in water storage containers, 9,528 home-made mosquito gravid oviposition sticky traps made from re-cycled plastic bottles, and empty container disposal practices to reduce mosquito breeding at households. The results show an increase in household water containers having fish from 11% to 42%, and a 51% reduction in number of adult mosquitoes in the intervention arm within one year compared to the control arm, with a 56% reduction in Container Index in the Intervention Arm compared to the Control Arm. The success and sustainability of these interventions is contingent upon effective community participation and cross-sectoral collaborations following the rationale offered by socio-ecological systems and resilience strategies. We facilitated capacity building within 100 school-teachers and 94 Community Health Workers to effectively harness support and foster experiential learning of school students and household members. This community participation enabled a better understanding of the underlying causes, transmission, and remedial actions to mitigate dengue. Examples of similar interventions in the GMS are discussed and expansion of community-driven initiatives explored.

119

ESTIMATING DENGUE TRANSMISSION INTENSITY FROM SEROLOGICAL DATA: A COMPARATIVE STUDY OF METHODS

Victoria Cox[1], Natsuko Imai[1], Megan O'Driscoll[2], Ilaria Dorigatti[1]

[1]*Medical Research Council Centre for Global Infectious Disease Analysis, Department of Infectious Disease Epidemiology, Imperial College London, London, United Kingdom,* [2]*Mathematical Modelling of Infectious Diseases Unit, Institut Pasteur, Paris, France*

Dengue infection is caused by dengue virus (DENV), an arbovirus with four antigenically distinct serotypes. DENV is a global health concern of increasing magnitude. To target the implementation of intervention strategies such as vaccination programmes or vector control measures, accurate estimates of DENV transmission intensity and age-specific seroprevalence are necessary. We compared the use of catalytic and mixture models to estimate DENV transmission intensity and the population seroprevalence. Catalytic models use thresholds to binarily classify serology data (antibody titres) as seropositive, indicating a previous infection, or seronegative. Historically these models have been favoured to estimate transmission intensity. However, a criticism of this method is that individual titres may be misclassified, leading to biased estimates. Mixture models are statistical models that can be applied to the full distribution

of individual antibody titres, without the need to binarily classify the data. There has not been significant application of mixture models in the context of DENV transmission. We applied catalytic and mixture models to DENV serology data from Vietnam and Indonesia, to compare the transmission intensity and seroprevalence estimates. Both models gave consistent estimates. In areas where DENV is less endemic, the mixture model was better able to capture the shape of the relationship between seroprevalence and age. Whilst further comparison is needed, these results suggest that the use of mixture models should be considered when estimating transmission intensity in areas where DENV is less endemic, or where the transmission intensity is lower.

120

INTERROGATING THE PRIMARY DENGUE-SPECIFIC MEMORY B CELL FOUNDER POPULATION IN HUMANS FOLLOWING DENGUE INFECTION

Zoe L. Lyski[1], Jana Mooster[1], Bettie Kareko[1], Brian Booty[2], William Messer[1]

[1]*Dept. Molecular Microbiology and Immunology, Oregon Health and Science University, Portland, OR, United States,* [2]*Oregon Clinical and Translational Research Institute (OCTRI), Portland, OR, United States*

Dengue virus (DENV) is the most important vector-borne viral pathogen of humans worldwide. Upon DENV infection, naïve host B-cells expand, produce, and secrete DENV-specific antibodies (Abs) that recognize viral antigens. After viral clearance, some of these B-cells become long-lived plasma cells (LLPC) which secrete DENV-Abs into the serum, while others become DENV-specific memory B cells (MBCs) that remain in circulation, quiescent, but poised respond and expand on repeat infection. This MBC "founder" population is expected to play a critical role in broader DENV immunity that is established following second DENV infection. Here we describe a strategy to identify and quantify DENV-specific MBCs in humans following a single DENV-1 infection. Peripheral blood mononuclear cells (PBMCs) from primary DENV-1 immune donors <1-21 years post infection were evaluated by two complimentary experimental approaches to quantify the DENV-specific MBC population. In the first approach, Limiting dilution assay, PBMCs are serially diluted and stimulated *in vitro* to become antibody-secreting cells. The resulting antibodies are assessed for DENV-specificity by ELISA using whole DENV virus (DENV1-4) and/or NS1. The second approach utilizes antigen-specific flow cytometry to quantify human MBCs (CD3-CD14-CD19+CD27+IgD-) that bind fluorescently labeled DENV-1. These DENV-specific MBCs are then single cell sorted and cultured along with cytokines and feeder cells to promote proliferation and antibody production. MBCs that secrete DENV-specific Abs undergo RT-PCR and cloning into human IgG expression plasmids for monoclonal antibody (mAb) production. Using these approaches, we identified DENV-whole virus and NS1-specific MBCs that remain in circulation decades after infection with varying frequencies. MBC-derived mAbs were then characterized for DENV-binding and neutralization. These experiments lay the foundation to characterize DENV-specific MBCs and functionally assess the antibodies they are programmed to secrete. The results of this project will provide insight into the MBC founder population following single DENV infection.

121

DEVELOPMENT OF HEK 293 CELL LINES CONSTITUTIVELY EXPRESSING FLAVIVIRAL ANTIGENS FOR USE IN DIAGNOSTICS

Jordan A. Powers, Benjamin Skinner, Lucy Robb, Elizabeth Gordon, Brent Davis, Amanda Calvert, Gwong-Jen J. Chang

Centers for Disease Control and Prevention, Fort Collins, CO, United States

Flaviviruses represent some of the most important human pathogens in the world. Diagnostic testing for these viruses is difficult since many of the pathogens require specialized biocontainment. Previously, we developed COS-1 cell lines secreting virus-like particles (VLPs) to produce non-infectious flaviviral antigen for diagnostic use, which are distributed to public health labs worldwide. While these VLP-secreting COS-1 cell lines have changed the landscape of diagnostic reagent production and distribution, their practicality is limited since secretion of VLPs tend to wane over several passages. To address this issue, we have generated 26 flaviviral VLPs and non-structural protein 1 (NS-1) secreting stable cell lines in HEK-293 cells to 13 different viruses including dengue viruses 1-4 (DENV 1-4), yellow fever virus (YFV), Japanese encephalitis virus (JEV), West Nile virus (WNV), St. Louis encephalitis virus (SLEV), Zika virus (ZIKV), Rocio virus (ROCV), Ilheus virus (IHLV), Usutu virus (USUV), and Powassan virus (POWV). DNA plasmids encoding the JEV signal sequence aids in the formation and secretion of VLPs that include pre-membrane (PrM) and envelope (E) structural proteins. These unique cell lines are grown in suspension with serum-free chemically defined media and no antibiotic selection. Antigen secretion was determined to be stable over 10 passages by ELISA where endpoints fluctuated no more than 4-fold, and immunofluorescent imaging (IFA) where percent positivity of the population of cells was greater than 70%. HEK-293 cell lines demonstrated higher secretion over 40-50 passages, whereas some COS-1 cell lines tended to decrease in percent positivity as measured by IFA and secretion endpoints within 10-20 passages of the cell line. These antigens are currently being evaluated for inclusion in CDC's clinical serological diagnostic formats. As shown here, development of VLPs as a platform to replace live or inactivated virus in diagnostic testing can be applied to medically relevant viruses.

122

"I COULDN´T STAND THE FEVER ANYMORE. IT FELT LIKE MY HEAD WAS GOING TO EXPLODE": A QUALITATIVE STUDY EXPLORING THE EXPERIENCE OF DENGUE-ASSOCIATED FEBRILE ILLNESS

Morgan A. Marks[1], Jane R. Wells[2], Amy M. Jones[2], Adam Gater[2], Shawna R. Calhoun[1], Beth-Ann Coller[1], Kevin Russell[1], Amy C. Morrison[3], Valerie A. Paz-Soldan[4], Tim Endy[5], Stephen Thomas[5], Todd Saretsky[1], Charlotte Panter[2]

[1]*Merck & Co. Inc, Kennilworth, NJ, United States,* [2]*Adelphi Values, Patient Centered Outcomes, Bollington, United Kingdom,* [3]*University of California, Davis, CA, United States,* [4]*Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States,* [5]*State University of New York, Upstate Medical University, Syracuse, NY, United States*

Dengue, a mosquito-borne viral infection, causes febrile illness that typically resolves within 14 days. Research studies to evaluate the clinical course of dengue tend to use a two-visit approach with initial visit at symptom onset and a visit after disease resolution. This design limits the ability to understand the spectrum of dengue signs/symptoms over the course of the illness. This qualitative study describes non-hospitalised participants' experience of dengue. Findings are being used to develop a patient-reported outcome measure assessing dengue. Adults and adolescents living in Iquitos, Peru with laboratory-confirmed dengue within the prior month were identified through community and clinic-based screening and approached for a face-to-face 60-minute interview. Interviews explored participants' dengue symptom experience and health-related quality of life and were conducted in Spanish by trained local researchers using a semi-structured interview guide. Interviews were audio recorded, transcribed, translated and analysed to identify common themes. Overall, 23 participants (14 female) were interviewed, 13 adults (aged 18-51 years) and 10 adolescents (aged 13-16 years). Most commonly reported symptoms included fever (100%), headache (96%), body pain (96%), eye pain (87%), chills (83%). Skin rash/itch, gastrointestinal symptoms (nausea, vomiting, abdominal pain, diarrhoea), tiredness, weakness, loss of appetite and a bad taste in the mouth were reported by at least 25% of participants. Symptoms lasted 8 days on average (3-15 days). Fever and headache were initial symptoms and rash/itch the last to resolve. The illness limited participants' ability to do housework, walk around, and get out of bed, which impacted emotional wellbeing (e.g. feeling sad, angry). This study identified aspects of dengue uncommonly reported, including bad taste in the mouth and impacts on daily activities. Understanding

the dengue experience is critical to inform development of measures of individual-level impacts of dengue which may assist with public health monitoring, vector control and vaccine clinical endpoint development.

123

SEROPREVALENCE AND DENGUE EPIDEMIOLOGY IN EIGHT DENGUE ENDEMIC COUNTRIES

Shibadas Biswal[1], Inge Lefevre[2], Vianney Tricou[2], Astrid Borkowski[2], TIDES Study Group

[1]*Takeda Vaccines, Inc, Cambridge, MA, United States,* [2]*Takeda Pharmaceuticals International AG, Zurich, Switzerland*

The ongoing TIDES trial evaluating efficacy of Takeda's tetravalent dengue vaccine (TAK-003) enrolled 20,099 healthy 4–16 year-olds living in endemic countries in Latin American (Brazil, Colombia, the Dominican Republic, Nicaragua, Panama) and Asia (the Philippines, Sri Lanka, Thailand) between September 2016 and March 2017. After sampling pre-vaccination, all participants have been under active surveillance for over 2 years of this nearly 5-year trial for febrile illnesses (defined as body temperature ≥ 38°C in any 2 of 3 consecutive days). Dengue cases are confirmed by a serotype-specific RT-PCR of the acute samples. One third of participants were randomized into the placebo group and are providing important data on dengue seroprevalence and epidemiology in the participating countries in a controlled setting of a phase 3 study. Of the 6684 placebo group participants evaluated at baseline for dengue neutralizing antibodies, 72.6% were seropositive (had reciprocal neutralizing titer ≥ 10) against one or more dengue serotypes; 59.9% were seropositive to all serotypes. A total of 6020 febrile illnesses were reported in the placebo group by 42.8% of the participants who experienced one or more episodes over the first ~27 months of follow up. Investigators reported 252/6008 (4.2%) cases as dengue fever and 33/6008 (0.5%) cases as dengue hemorrhagic fever based on clinical assessment. A total of 312 dengue cases were confirmed in the placebo group by RT-PCR in this period with an overall incidence of 2.1 cases (95% CI: 1.9–2.4) per 100 person years; 2.6 (2.0–3.5) in the 4–5 years, 2.3 (2.0–2.7) in the 6–11 years, and 1.6 (1.2–2.0) in the 12–16 years age groups. Analysis by baseline serostatus showed an incidence per 100 person years of 2.1 (1.7–2.6) in dengue-naïve and 2.1 (1.9–2.4) in dengue pre-exposed participants. There were 91 dengue cases requiring hospitalization, an incidence of 0.6 (0.5–0.8) cases per 100 person years. Further analysis of data by country has been performed and will be presented. The remaining duration of the trial will generate additional valuable data on dengue epidemiology in the 8 participating countries.

124

INFECTION OF HUMAN IPS CELL- AND/OR PERIPHERAL CD14$^+$ CELL-DERIVED AND IMMORTALIZED MYELOID CELL LINES (MYLC) WITH DENGUE VIRUS

Jun Shimizu[1], Tadahiro Sasaki[2], Atsushi Yamanaka[3], Naomi Tanga[1], Yuka Yoshimura[1], Ami Murakami[1], Ritsuko Koketsu[2], Yoshihiro Samune[2], Kazuo Miyazaki[1], Emi Nakayama[2], Tatsuo Shioda[2]

[1]*MiCAN Technologies Inc., Kyoto, Japan,* [2]*Research Institute for Microbial Diseases, Osaka University, Osaka, Japan,* [3]*Mahidol-Osaka Center for Infectious Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand*

Virus isolation from patient's specimens is the most reliable evidence for the diagnosis of viral infections. Although many kinds of cells are used for virus isolation, the sensitivity of each cell to virus varies greatly depending on the virus species. For example, Vero cells or C6/36 cells, which are not human origin, are generally used for dengue virus isolation. We have a platform to establish immortalized myeloid cell lines from human iPS cells and/or primary CD14$^+$ cells purified from human peripheral blood mononuclear cells (PBMC). In this study, using this platform, we have established several human myeloid cells lines (Mylc lines) and examined whether these Mylc lines can be infected with dengue virus type 2 (DV2) 16681 strain. First, we analyzed infectivity of two DV2 stocks propagated in C6/36 cells (DV2(C6)) or Vero cells (DV2(Vero)). Infectivity of DV2(C6) to Vero cells was less efficient than that of DV2(Vero). However, after passaging of DV2(C6) on Vero cells five times, the virus showed tendency towards increased infectivity to Vero cells. This result indicated that cell types for viral propagation could significantly affect the infectivity of DV2. We next examined whether Mylc lines before and after the differentiation into dendritic cell (DC) can be infected with DV2(Vero) or DV2(C6). Mylc lines can be differentiated into DCs by culturing Mylc lines along with IL-4 for 3 days. Interestingly, almost of all Mylc lines exhibited no infection with DV2(C6), and all Mylc lines could be infected with DV2(Vero), especially after DC-differentiation (7 out of 7 lines examined). One of DC-differentiated Mylc lines showed increased or equivalent sensitivity to both DV2(Vero) and DV2(C6) compared with Vero cells. Also, we found Mylc lines maintained in one particular medium (DCO-K medium) exhibited significantly enhanced infectivity compared with regular medium (MEM alpha). Therefore, these results indicate that human Mylc lines may be useful and powerful tool for virus isolation and research, and that it may be possible to establish more sensitive Mylc lines than Vero cells using our platform.

125

VECTOR COMPETENCE OF NATURAL FLORIDA *AEDES AEGYPTI* TO A REGION-SPECIFIC DENGUE 4 VIRUS ISOLATE

Caroline J. Stephenson, Heather G. Coatsworth, John A. Lednicky, Rhoel R. Dinglasan

University of Florida, Gainesville, FL, United States

Dengue viruses (DENVs) are responsible for the largest number of arbovirus infections among humans globally. In the state of Florida (FL), USA, sporadic yearly DENV transmission has occurred since 2009. *Aedes aegypti* are the principal DENV vector and their ability to acquire and transmit the virus (their vector competence) is found to be affected by factors relating to the vector, the virus and the environment. Few studies have examined the vector competence of field derived FL *Ae. aegypti* for dengue viruses. DENV-4 was detected in and sequenced from *Ae. aegypti* F1 adult mosquitoes reared from eggs collected from four West-Central Florida sites in 2016-2017. Notably, no human index case was reported across both years, suggesting its vertical maintenance. We hypothesize that natural *Ae. aegypti* populations from Southern FL will be highly susceptible and competent to a recent DENV-4 isolate from Haiti, a region contributing to a high volume of imported cases in FL. We also predict that a 1952 derived laboratory colony of *Ae. aegypti* from Orlando (ORL), FL will exhibit comparable vector competence, offering itself as a useful model to test vector competence across various dengue virus strains. Four our approach, adult *Ae. aegypti* from Collier County in Southwestern FL and ORL are infected *per os* with either DENV-4 Haiti or laboratory strain (H241) and virus presence is detected in the midgut and saliva 14 days post-infection by RT-PCR. Preliminary day 14 midgut infection rates (IRs) for DENV-4 Haiti were 43% for Collier mosquitoes and 83% for ORL. Midgut IRs for DENV-4 H241 were 50% for Collier and 38% for ORL. Approximately 15% of ORL and 30% of Collier mosquitoes had detectable virus in their saliva for both DENV-4 groups. Further studies will quantify virus titers in these tissues via plaque assay and examine DENV-4 vector competence across *Ae. aegypti* from Northern, Central and Southern FL. Our goal is to better understand where vector competency levels are high enough to increase the risk of transmission to humans. Importantly, such data would allow us to predict and proactively control future dengue transmission in this state.

126

MAST CELL TRYPTASE AS A PREDICTIVE BIOMARKER FOR DEVELOPING SEVERE DENGUE

Robert D. Hontz[1], Diego D. Galan[2], Tyler Warkentien[1], Chinmay K. Mantri[3], Jenny Low[4], Ashley L. St. John[3]

[1]*NAMRU-2, Singapore, Singapore,* [2]*Duke University, Durham, NC, United States,* [3]*Duke-NUS Medical School, Singapore, Singapore,* [4]*Singapore General Hospital, Singapore, Singapore*

Dengue disease is endemic to most tropical and sub-tropical countries worldwide, impacting hundreds of millions every year. Dengue disease progresses from mild symptoms "with or without warning signs" (dengue fever (DF)) to life-threatening "dengue hemorrhagic fever" (DHF). Earlier data from Duke-National University of Singapore (Duke-NUS) among Bangladeshi patients indicated that elevated chymase, a mast cell (MC) protease, was predictive of later onset of DHF in children and adults. In this study, another MC protease, tryptase, a regulator of vascular leakage, was investigated to determine the extent of its association with hemorrhagic complications of dengue. Here, we assessed the correlation between tryptase levels and signs of vascular leakage in prospectively recruited patients in Singaporean hospitals and clinics. Patients were sub-divided into groups based on outward signs at initial presentation: "bleeding" and "non-bleeding." The "bleeding" group showed average plasma tryptase concentrations of 385.31 pg/mL, compared to 326.61 pg/mL for the "non-bleeding" group (*t*-value=1.86; p=0.035); demonstrating statistically significant associations between elevated tryptase and vascular complications of dengue. Secondarily, immune cell counts were measured between both groups, showing that basophils were the only immune cell-type significantly higher (by *t*-test) in patients that experienced bleeding. Regression analysis detailed no significant correlation between basophil counts and serum tryptase levels. Although subject enrollment is still ongoing, but is soon to be concluded, results indicate a correlation between higher serum tryptase levels and bleeding symptoms, making tryptase a promising biomarker for early detection of severe dengue disease. Such a target would be extremely promising for future development into an easy-to-use handheld or diagnostic device for early detection of dengue in resource-poor settings or austere/remote environments.

127

KNOWLEDGE, ATTITUDES AND PRACTICES ON DENGUE AND IT'S PREVENTION AND CONTROL AMONG SCHOOL CHILDREN IN THE DISTRICT OF KALUTARA, SRI LANKA

Thilanka Bandara[1], Mizaya Cader[2], D S Anoja F Dheerasinghe[3]

[1]*National Dengue Control Unit, Colombo, Sri Lanka,* [2]*National Programme for Tuberculosis Control and Chest Diseases, Colombo, Sri Lanka,* [3]*Office of the Provincial Director of Health Services – Western Province, Colombo, Sri Lanka*

Dengue affects 390 million people worldwide, 70% of whom live in Asia. It is hyper-endemic in Sri Lanka, with ≈30% of affected patients being of school-going age. Considering the school children as good change agents, many curricula and behavioural related interventions have been implemented in schools to improve the knowledge, attitudes and practices on dengue among schoolchildren to empower them to assist sustainable dengue prevention and control in the country. The objective of the study was to describe the knowledge, attitudes and practices on dengue and its control among grade 9 government school children in Horana Educational Zone in the District of Kalutara, Sri Lanka. A descriptive cross-sectional study was conducted among 782 grade 9 students in Horana Educational Zone. The participants were selected using a multi-stage cluster sampling technique with probability proportionate to population size. A pre-tested, structured, self-administered questionnaire was used for the assessment, after ensuring face and content validity by experts. The scoring system was prepared after weighting of the questionnaire by five independent experts in the field. The majority (85.7%; 95%CI= 83.1-88.1) of the schoolchildren demonstrated adequate knowledge on dengue and its control. Further, the overall attitude on dengue control in relation to the burden, human behaviour and source reduction was good among 85.7% (95%CI=83.2-88.0) of the participants. Overall; the reported practices were good among 86.2% (CI=83.6-88.5) with a good understanding of the dynamics of dengue mosquito vectors. Grade 9 government school children in Horana Educational Zone had adequate knowledge on dengue and dengue control, good attitude towards dengue control, and good reported practices on prevention and control of dengue.

128

IMPACT OF PRIOR FLAVIVIRUS VACCINATION ON IMMUNOGENICITY AND EFFICACY OF TAKEDA'S PURIFIED INACTIVATED ZIKA VACCINE IN INDIAN RHESUS MACAQUES

Ginger Young[1], Melissa Zahralban-Steele[1], C. Steven Godin[2], Hetal Patel[1], Hansi Dean[1]

[1]*Takeda Vaccines, Inc., Cambridge, MA, United States,* [2]*Inotiv, Gaithersburg, MD, United States*

Cocirculation and coinfection of vector-borne flaviviruses in endemic areas can create a cross-reactive immune response in individuals infected with one or more flaviviruses. In addition, flavivirus vaccinations, including Yellow Fever (YFV), Japanese Encephalitis (JEV), West Nile virus (WNV), or Tick Borne Encephalitis (TBE) vaccines, may influence immune responses to other flavivirus vaccines. We evaluated the immunogenicity and efficacy of Takeda's purified inactivated Zika vaccine (PIZV) candidate in Indian rhesus macaques previously vaccinated with commercial YFV, JEV, WNV, or TBE vaccines. Six months following flavivirus vaccination, macaques were vaccinated with two doses of PIZV 28 days apart. Macaques vaccinated with PIZV alone or with YFV, WNV, or TBE vaccines followed by PIZV were challenged with wild type Zika virus (ZIKV) strain PRVABC59 eight months after the second PIZV dose. Sera were collected monthly throughout the study to evaluate immunogenicity. After ZIKV challenge, viral RNA was measured to assess vaccine efficacy. We determined that previous vaccination with YFV, JEV, WNV, or TBE had no impact on the ZIKV neutralizing antibody responses induced by PIZV. All challenged macaques were protected against ZIKV infection, indicating that vaccine-induced immunity against YFV, WNV, or TBE does not impact PIZV efficacy.

129

HEPATITIS C VIRUS (HCV) INFECTION AMONG SICKLE CELL DISEASE PATIENTS AT THE KORLE- BU TEACHING HOSPITAL

Gifty Mawuli[1], Bartholomew Dzudzor[2], Kenneth Tachi[3], Amma Anima A. Benneh-Akwasi Kuma[4], James Odame-Aboagye[1], Billal O. Obeng[5], Anthony T. Boateng[1], Elijah P. Edu-Quansah[6], Keren O. Attiku[1], Esinam Agbosu[1], Augustina Arjarquah[1], Joseph H. Bonney[1]

[1]*Noguchi Memorial Institute for Medical Research, Accra, Ghana,* [2]*Department of Medical Biochemistry, School of Biomedical and Allied Health Sciences, University of Ghana, Accra, Ghana,* [3]*Department of Medicine and Therapeutics, College of Health Sciences, University of Ghana, Accra, Ghana,* [4]*Department of Haematology, College of Health Sciences, University of Ghana, Accra, Ghana,* [5]*Department Medical Microbiology- School of Biomedical and Allied Health Sciences, Accra, Ghana,* [6]*African Field Epidemiology Network, Liberia, Accra, Liberia*

Hepatitis C virus (HCV) infection is a blood borne infection that remains potentially transmissible through blood transfusions. Sickle cell disease (SCD) is a common heritable haemoglobinopathy in Ghana that is often treated with multiple blood transfusions. The SCD patient is therefore at high risk of HCV infection; however, data on the occurrence of HCV in SCD patients has not been documented in Ghana. This study sought to determine the prevalence and genotypes of HCV infection in sickle cell anemia patients. This was a cross-sectional study which enrolled 142 sickle-cell anemia patients from the Ghana Institute for Clinical Genetics, Korle-Bu Teaching Hospital. Patient information was obtained through a questionnaire and 3mls of whole blood was collected. Aliquots of the plasma obtained was used for both serology and molecular testing by RT-PCR with primers targeting the HCV core gene. The amplified DNA

were purified and subjected to phylogenetic analysis to characterize HCV genotypes. Out of the 142 patients' samples collected 12 (9%) were sero-positive for anti- HCV total antibodies. HCV RNA was amplified from 8 (6%) out of the 142 patients' samples. One of the 12 sero-positives was HCV RNA positive. Five (63%) out of the 8 HCV RNA positive samples were successfully sequenced. The phylogenetic tree constructed with the study and GenBank reference sequences, clustered all five study sequences into HCV genotype 1. The HCV seroprevalence of 9% among sickle cell patients is higher than reported for the general population. Genotype 1 is the common HCV genotype infecting SCA patients. Sickle cell anaemia is a high-risk group for HCV infection in Ghana. This group therefore must be given priority in resource allocation for preventive, diagnostic and therapeutic strategies.

130

ROLE OF THE MOSQUITO SALIVARY PROTEINS IN ZIKA VIRUS INFECTION

Paola Valenzuela

NIH, Rockville, MD, United States

The Zika virus is transmitted to humans by female *Aedes aegypti* and *Aedes albopictus mosquitoes*, which inoculate virus into the dermis and epidermis while feeding. During the feeding process, infected mosquitoes deliver not only the virus into the skin but also biologically active salivary components. Salivary proteins are known to help the mosquito to get a blood meal by inhibiting the blood coagulation cascade, vasoconstriction, platelet aggregation, and other host response. Importantly, mosquito salivary components were shown capable of modifying the replication of arthropod-borne viruses. Although little information has been generated regarding the additional effects of the saliva of the vector on the infection with the Zika virus. We perform the expression and purification of 20 recombinant proteins in HEK-293 cells; these proteins are female-specific and secreted in the saliva of *Aedes aegypti* mosquitoes. Using surface plasmon resonance, we identified six proteins that can interact with the domain III and the complete Envelope protein of the Zika virus, suggesting their interactive role with the virus that further modulates the viral infection and host immune response. In addition, we have examined the contribution of the salivary gland extract (SGE) of *Aedes aegypti* mosquitoes in the antibody-dependent enhance (ADE) using K562 cells infected with Zika virus. More ADE in the presence of SGE was observed *in vitro*. However, a positive correlation between the levels of antibodies IgG1 and IgG4 that recognize the NIH435-9 protein (a protein that binds to the envelope protein with high affinity), and IgG antibodies that recognize the non- structural protein 1 (NS1) of the Zika virus was found. Our data suggest that the Zika virus is released in the skin coated with proteins that are present in the saliva of mosquitoes and not alone, and antibodies against these proteins could have a potential role in modulating the transmission and infection of the virus during the antibody-dependent enhancement.

132

YELLOW FEVER VIRUS VACCINATION ANTIBODIES ENHANCE ZIKA VIRUS INFECTION IN EMBRYOID BODIES

Emily M. Schultz, TyAnthony J. Jones, Kelli L. Barr

Baylor University, Waco, TX, United States

Zika virus (ZIKV) is a flavivirus that originated in Africa but emerged in the Western Hemisphere in 2015. In these areas, other flaviviruses such as Dengue (DENV) and West Nile virus co-circulate. Moreover, vaccination for Yellow Fever Virus (YFV) is widely practiced. Studies have found antibody mediated enhancement between DENV and ZIKV, but the impact of YFV antibodies on ZIKV infection has not been fully explored. Since ZIKV infections cause congenital syndromes, such as microcephaly, the impact of cross-reactive antibodies on transmission of ZIKV through the placental barrier needs to be characterized. Recent advancements in biomedical engineering have generated cellular co-culture models that allow for *in vitro* replication of the maternal-fetal interface. This study utilized a co-culture model with human placental syncytiotrophoblasts, human fetal umbilical cells, and differentiating human embryoid bodies. We employed a collagen-coated PTFE membrane with maternal cells on the apical side of the membrane and fetal umbilical cells on the basolateral side in order to replicate the maternal-fetal axis. To determine if YFV vaccination antibodies impact the replication and movement of ZIKV across the maternal fetal axis, maternal syncytiotrophoblasts were innoculated with ZIKV or ZIKV incubated with YFV-17D post vaccination serum collected at various time points following vaccination. Viral load was measured 72 hours post inoculation. The data show that the impact of YFV vaccine antibodies on ZIKV replication is cell line dependent. In embryoid bodies, the presence of YFV vaccine antibodies enhanced ZIKV infection with the greatest enhancement produced with YVF vaccinate serum collected between 120-420 days post vaccination. Since viral pathogenesis, and the impact of antigenic cross-reactive antibodies, is cell line specific at the maternal-fetal axis, this suggests alternative avenues for addressing the impact of congenital ZIKV infections. Further, this study highlights the potential impact of cross-reactive antibodies from YFV vaccines on ZIKV congenital infections.

133

IMPACT OF HEMATOPHAGY ON ANTIMICROBIAL RESPONSES IN MOSQUITOES

Bailey Maloney[1], Yan-Jang Huang[1], Ashley Bilyeu[1], Amy Lyons[1], Wei-Wen Hsu[2], Stephen Higgs[1], Dana Vanlandingham[1]

[1]*Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, United States,* [2]*Department of Statistics, College of Arts and Sciences, Kansas State University, Manhattan, KS, United States*

A majority of pathogenic flaviviruses are transmitted by mosquitoes in the *Culicinae* subfamily. These mosquito-borne flaviviruses initiate infections in competent mosquito species through engorgement of infectious blood meals. Whilst it has long been known that hematophagy is a critical step for the establishment of flavivirus infections; questions such as how blood feeding can impact the antiviral immunity of mosquitoes and the effect of blood feeding in the establishment of effective flavivirus infections have rarely been examined. In this study, meals consisting of either defibrinated sheep blood or bovine serum albumin protein solutions were used to evaluate the effects of hematophagy on antimicrobial responses in *Aedes* and *Culex* species mosquitoes. Significant differences in expression levels of Cactus and Caspar genes were observed between mosquitoes receiving blood and protein meals. These findings are consistent with previous reports of differences between infectivity of flaviviruses presented through infectious blood and protein meals. Our observations suggest that the immunological responses of mosquitoes may be modulated by blood feeding. The feasibility of applying this model to the investigation of vector-host interactions that modulate antiviral response mechanisms and the kinetics of flavivirus infections in mosquitoes is supported by the results of our study.

134

HETEROLOGOUS FLAVIVIRUS EXPOSURE PROVIDES VARYING DEGREES OF CROSS-PROTECTION FROM ZIKA VIRUS DISEASE IN A MOUSE MODEL OF INFECTION

Mariah Hassert[1], Tara Steffen[1], Bryce G. Dorflinger[2], Jacquelyn Dayton[2], Abigail Coleman[1], Lillian Cruz-Orengo[2], James D. Brien*[1], Amelia K. Pinto*[1]

[1]*Saint Louis University, St. Louis, MO, United States,* [2]*University of California-Davis, Davis, CA, United States*

The 2016 Zika virus (ZIKV) epidemic in South and Central America left the scientific community pushing to understand the disease, and importantly, the critical factors which modulate protection from this pathogen. A number of studies have highlighted that having been exposed to a heterologous flavivirus may modulate the immune response to ZIKV. However, it is still unclear 1) how this impacts viral burden and pathology,

and 2) the discrete factors which correlate with cross-protection. We hypothesize that due to genetic and antigenic similarities, that prior exposure to a related flavivirus will lead to reduced disease severity, mortality, and viral burden during ZIKV infection. In this murine study, we longitudinally examine multiple factors involved in flavivirus cross-protection, linking flavivirus immune status to ZIKV disease severity, viral burden and neuropathology. We show that while heterologous flavivirus exposure with dengue virus 2 or 3, or the vaccine strain of yellow fever, provides protection from mortality in a lethal ZIKV challenge, reduction in viral burden and disease varies depending on the infecting primary virus; with primary ZIKV infection being most protective, followed by dengue 2, yellow fever, and dengue 3. We not only evaluate this cross-protection by survival, weight loss, and viral burden, but also link physical indicators of neurological disease severity with multiple histological metrics including neurophagia, astrogliosis and microgliosis within the cerebral cortex. Overall, this study demonstrates a protective impact of prior heterologous flavivirus exposure on ZIKV pathogenesis and will potentially inform the next generation of flavivirus vaccines.

135

ZIKA VIRUS REPLICATES IN THE LOWER FEMALE REPRODUCTIVE TRACT OF IMMUNE-COMPETENT MICE

Cesar A. Lopez, Helen M. Lazear

University of North Carolina at Chapel Hill, Carrboro, NC, United States

Zika virus (ZIKV) is the first example of an arbovirus that is transmitted between humans through sexual contact, requiring the virus to surmount mucosal immune defenses not encountered during mosquito-borne transmission. Although ZIKV can replicate in immune-competent primates, the virus replicates poorly in immune-competent mice (e.g. C57BL/6J), likely because ZIKV fails to antagonize murine STAT2 and STING, key components of the antiviral type I interferon (IFN) response. Accordingly, ZIKV mouse models typically use mice with impaired IFN signaling (e.g. Ifnar1-/- or treatment with an IFNAR1-blocking antibody). Thus, it was unexpected when we detected productive ZIKV replication after intravaginal inoculation of wild-type C57BL/6J mice. We detected increasing ZIKV levels by qRT-PCR in vaginal washes for 8 days followed by clearance, consistent with an adaptive immune response. Although viral loads in the vagina increased in WT mice, we detected viremia and infection of the upper reproductive tract only in Ifnar1-/- mice, not WT mice. Productive vaginal infection requires pre-treatment with medroxyprogesterone (DMPA) but DMPA did not render WT mice more susceptible to subcutaneous ZIKV inoculation, suggesting a specific effect in the female reproductive tract, rather than systemic immune suppression. Other flaviviruses (e.g. Spondweni virus and Usutu virus) also were able to replicate in the lower female reproductive tract of WT mice, indicating that permissiveness to viral infection at this site is not specific to ZIKV. RNA *in situ* hybridization imaging revealed ZIKV RNA throughout the vagina of infected mice, both in the epithelium and the underlying stroma. Ongoing studies seek to understand the immune mechanisms that restrict systemic but not lower female reproductive tract ZIKV infection, as well as the potential for sexually transmitted ZIKV to result in ascending congenital infections.

136

DEVELOPMENT OF A SMALL ANIMAL MODEL FOR DEER TICK VIRUS PATHOGENESIS MIMICKING HUMAN CLINICAL OUTCOME

Meghan E. Hermance[1], Charles E. Hart[2], Allen T. Esterly[2], Erin S. Reynolds[2], Jahnavi R. Bhaskar[2], Saravanan Thangamani[2]

[1]*University of South Alabama, Mobile, AL, United States,* [2]*SUNY Upstate Medical University, Syracuse, NY, United States*

Powassan virus (POWV) is a tick-borne flavivirus that encompasses two genetic lineages, POWV (Lineage I) and deer tick virus (DTV, Lineage II). In recent years, the incidence of reported POWV disease cases has increased, coupled with an expanded geographic range of the DTV tick vector, *Ixodes scapularis*. POWV and DTV are serologically indistinguishable, and it is not known whether clinical manifestations, pathology, or disease outcome differ between the two viruses. In the present study, six-week-old male and female BALB/c mice were footpad-inoculated with DTV doses ranging from 10^1 to 10^5 FFU. Dose-independent mortality, morbidity, and organ viral loads were observed for mice inoculated with sequentially increasing doses of DTV. By study completion, all surviving mice had cleared their viremias but detectable levels of negative-sense DTV RNA were present in the brain, indicating viral persistence of infectious DTV in the central nervous system. For mice that succumbed to disease, neuropathology revealed meningoencephalitis characterized by microscopic lesions with widespread distribution of viral RNA in the brain. These findings, coupled with the rapid onset of neurological signs of disease and high viral titers in nervous tissue, highlight the neurotropism of DTV in this mouse model. Additionally, disease outcome for DTV-infected mice was not affected by sex, as males and females were equally susceptible to disease. This is the first study to comprehensively characterize the clinical disease outcome in a small animal model across a spectrum of POWV/DTV infection doses. Here, we developed a small animal model for DTV pathogenesis that mimics the manifestations of POWV disease in humans. Since it is currently not known whether DTV and POWV differ in their capacity to cause human disease, the animal model detailed in our study could be utilized in future comparative pathogenesis studies, or as a platform for testing the efficacy of vaccines, and anti-virals.

137

A HUMAN SKIN MODEL FOR ASSESSING THE ROLE OF SALIVARY FACTORS ON VIRUS TRANSMISSION AT THE MOSQUITO-VIRUS-HOST INTERFACE

Allen T. Esterly, Megan Gribble-Llyod, Jennifer Moffat, Saravanan Thangamani

SUNY Upstate Medical University, Syracuse, NY, United States

Infectious agents transmitted by mosquitoes are delivered to vertebrate hosts with arthropod saliva at the bite site. Mosquito saliva has been demonstrated to impact host immune responses and enhance pathogen transmission. More specifically, as a virus is inoculated into the skin during mosquito feeding, vector salivary components modulate host cell responses facilitating skin infection and subsequent virus dissemination within the host. To advance our understanding of the complex cellular interactions at the mosquito-virus-host interface, we have developed a novel human skin ex-plant method. Utilizing human skin ex-plants in culture, we demonstrate the ability to investigate the impact of mosquito salivary factors modulating immune responses, cellular interactions, and virus replication. This skin ex-plant model, derived from human breast tissue, consists of complete epidermal and dermal tissues which provide a full range of cutaneous cell types. This method will give us unparalleled insight into a mosquito-virus-host-like interface which single cell culture methods lack due to inherit bias of a particular cell type. This approach provides an alternative methodology to using model organisms of infection but also provides a cost-effective strategy to immunologically characterize mosquito-borne viruses with a higher degree of accuracy since our representative site of interaction is of human origin.

138

EFFICACY OF HUMAN POLYCLONAL ANTIBODIES TO ZIKA VIRUS (ZIKV-IG) IN PREGNANT NON-HUMAN PRIMATE MODEL

Hongyu Qiu[1], Dawn Dudley[2], Keisuke Yamamoto[2], Phoenix Shepherd[2], Meghan Breitbach[2], Christina Newman[2], Mason Bliss[3], Sierra Rybarczyk[3], Michelle Koenig[4], Michelle Schotzko[3], Emily Sneed[3], Maria Dennis[5], Heather Simmons[3], Andres Mejia[3], Michael Fritsch[2], Matthew Aliota[6], Thomas Friedrich[7], Sallie Permar[5], David O'Connor[2], Barker Douglus[1], Shantha Kodihalli[1]

[1]*Research and Development, Therapeutics, Emergent BioSolutions Canada, Winnipeg, MB, Canada,* [2]*Department of Pathology and Laboratory*

Medicine, University of Wisconsin-Madison, Madison, WI, United States, [3]Wisconsin National Primate Research Center, University of Wisconsin-Madison, Madison, WI, United States, [4]Department of Comparative Biosciences, University of Wisconsin-Madison, Madison, WI, United States, [5]Department of Pediatrics and Human Vaccine Institute, Duke University Medical Center, Durham, NC, United States, [6]Department of Veterinary and Biomedical Sciences, University of Minnesota, Saint Paul, MN, United States, [7]Department of Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI, United States

Zika virus (ZIKV) infection during pregnancy can cause severe outcomes such as Congenital Zika Syndrome (CZS), with 3,490 CZS cases confirmed during the 2015-2017 epidemic in the Americas. Currently there are no FDA approved treatments or vaccines to prevent or minimize the severity of CZS. To address this unmet need, Emergent has developed an Anti-Zika Immune Globulin (Human) (ZIKV-IG) product for prophylaxis of ZIKV infection in at-risk populations, including women of childbearing potential and pregnant women. A pregnant NHP model was used to assess the protection of ZIKV-IG when administered after infection. Eight pregnant rhesus macaques infected with 10^4 PFU ZIKV-PR at gestational day (GD) 45 were treated with either ZIKV-IG or non-specific IG (control) by *i.v.* infusion at 1 and 5 days post-infection (dpi) and monitored for circulating virus in plasma until 110 dpi. Virus was detected (10^3 to 10^4 RNA copies/mL plasma) of all animals treated with ZIKV-IG before the first dosing, and was undetectable by 2 dpi. One animal experienced viral recrudescence to 10^3 RNA copies/mL plasma at 41 dpi through 48 dpi. Control animals had detectable virus in the plasma for 5-13 days post-infection, with peak viremia of 10^4 to 10^6 RNA copies/mL plasma, after which the virus was no longer detectable. ZIKV-IG pharmacokinetics were determined with a whole virion binding ELISA. Fetal growth was normal by weekly ultrasound measurements for both treatment and control groups, and all fetuses survived to term (155 GD) with no gross abnormalities noted at necropsy. Pathology score of fetal tissues and maternal/fetal interface were not significantly different between ZIKV-IG and control groups. These tissues were also negative for ZIKV vRNA by qRT-PCR. The absence of ZIKV vertical transmission in the control groups could be due to the low rate of adverse outcomes with Zika infection in this model. The significant reduction of maternal viremia highlights the potential of ZIKV-IG for prevention of CZS.

139

SCREENING FOR AUTISM SPECTRUM DISORDER AMONG TODDLERS FROM THE PEDIATRIC OUTCOMES OF PRENATAL ZIKA EXPOSURE STUDY IN SOUTHERN PUERTO RICO

Viviana Rosario-Villafañe, Irelis C. Repollet-Carrer, Marilyn Borges-Rodríguez, Vanessa Rivera-Amill, Luisa I. Alvarado-Domenech, **Mary Rodríguez-Rabassa**

Ponce Health Sciences University, Ponce, PR, United States

Congenital infections, such as Congenital Rubella have been linked to developmental and behavioral problems that manifest early or develop over time, such as the Autism Spectrum Disorder (ASD). Given that Zika virus gestational infection is associated with developmental problems, it is possible that the same pathophysiologic mechanisms leading to ASD are also present in Congenital Zika. This study aims to describe the results of the Modified Checklist for Autism in Toddlers Revised (M-CHAT-R) and the Ages and Stages Questionnaires Social-Emotional-2 (ASQ-SE-2) of toddlers enrolled in the Pediatric Outcomes of Prenatal Zika Exposure (POPZE) cohort study. POPZE enrolled Zika exposed infants from two southern hospitals in Puerto Rico. The M-CHAT-R and ASQ-SE-2 are parent completed questionnaires: M-CHAT-R is used to identify risk for autism, and ASQ-SE-2 examines social and emotional development to identify needs for referral. Both were completed at least once during follow-up visits at 18, 24, or 30 months of age from March 2019 to March 2020. Newborn imaging results were obtained from the birth record. Descriptive statistics were used to analyze sociodemographic characteristics, clinical features at birth and questionnaire scores. Questionnaires were completed by 56 mothers and 28 (50%) infants were screened twice. Forty-eight (86%) mothers serve as primary caregiver. Thirty-four toddlers (61%) are female, and the majority (51%) were screened at their 30-month visit. M-CHAT-R scores of eight (10%) toddlers suggest they were at medium risk for autism. Among this group, four (50%) toddlers had optic nerve abnormalities at birth, and one (13%) had an abnormal brain ultrasound at birth. Six (75%) toddlers who demonstrated medium risk in M-CHAT-R had a score above cut-off in ASQ-SE-2, requiring a follow-up visit to confirm developmental risks. Screening toddlers who are at risk for neurologic and developmental abnormalities allows for more thorough developmental evaluations and timely referral. ASD early diagnosis and intervention can help mitigate adverse outcomes and may prevent secondary behavioral and developmental consequences.

140

DETECTION OF MONKEYPOX VIRUS IN AN OUTBREAK OF UNKNOWN ETIOLOGY USING NEXT GENERATION SEQUENCING

Amuri Aziza Adrienne[1], Catherine Pratt[2], Eddy Kinganda Lusamaki[1], Placide Mbala Kingebeni[1], Elisabeth Simbu Pukuta[1], Michael Wiley[2], Steve Ahuka Mundeke[1], Jean Jacques Tamfum Muyembe[1]

[1]*Institut National de Recherche Biomediacle, Kinshasa, Democratic Republic of the Congo,* [2]*University of Nebraska Medical Center, Omaha, NE, United States*

The Democratic Republic of the Congo has been responding to the country's 10th Ebola virus disease outbreak since August 2018. Between the 6th and 12th of January 2020, MoH were notified of a cluster of 12 cases with suspected Ebola virus disease in Kiri health zone. Seven samples were sent for Ebola virus testing at the Institut National de Recherche Biomédicale (INRB), in Kinshasa. All of the samples tested negative for Ebola virus. INRB scientists used their genome sequencing capacity, set up during June 2018 to aid the response to the ebola virus disease outbreak, to process the samples with an unknown etiology. A pan-viral targeted enrichment method was used to sequence the samples. Libraries were prepared with a KAPA RNA Hyperprep kit and IDT xGen Dual Index UMI adapters, then enriched with an Illumina RNA Exome Enrichment kit with the Twist Biosciences Pan-Viral probe panel. The enriched libraries were loaded on an Illumina iSeq for 2 x 151 cycles. The resulting fastq were de-novo assembled using an in-house pipeline. Three cases were determined to be positive for Monkeypox virus, with reads covering 3.4%, 5.6%, and 97.9% of the monkeypox genome. The presence of Monkeypox virus was confirmed by PCR. One further case was stongly positive for malaria. All seven cases had evidence of herpesvirus infections, including Epstein Barr virus, Parvovirus B19 and Cytomegalovirus, which were likely to be latent, rather than active infections. This genome sequencing effort resulted in the detection of an undiagnosed outbreak of Monkeypox in a region which had no recent cases of monkeypox virus. Nonspecific rash and febrile illnesses are a common cause of morbidity and mortality in low-resource countries, but are challenging to diagnose in these envrionments. In-country capacity building during high-profile disease outbreaks, rather than temporary parachute support, can provide durable and important capabilities after the high-profile outbreak has waned. INRB are now well-placed to use their sequencing capability to perform bio-surveillance to improve the understanding of the infectious disease burden in the DRC.

141

SECONDARY ATTACK RATE OF EBOLA VIRUS DISEASE 2019 IN BENI DEMOCRATIC REPUBLIC OF CONGO HOUSEHOLDS FROM JANUARY TO NOVEMBER 2019

Gisele M. Mvumbi[1], Vitus O. Okitolonda[1], Alfred T. Toi[2], Carolina S. Braga[3], Prince K. Kimpanga[1]

[1]*ESP-KINSHASA/DRC, Kinshasa, Democratic Republic of the Congo,* [2]*Université Laval, Quebec, QC, Canada,* [3]*Laval University, Quebec, QC, Canada*

Secondary attack rate of Ebola virus disease 2018 in Beni-Democratic Republic of Congo households, January - November 2019Understanding

the household transmission of the Ebola 2019 epidemic virus, including risk factors for transmission, is important to refine public health strategies to reduce the burden of disease. Over the period January to November 2019, we studied the transmission of the emerging virus in 1998 households in which the index case was the first symptomatic case of Ebola 2019. Secondary cases were defined as household contacts with a laboratory-confirmed case of Ebola virus disease (EVD 2018), occurring approximately 8-10 days later but within 21 days of the onset of symptoms in the index case. The objective was to estimate the secondary attack rate and to describe the characteristics of index cases and their household contacts associated with the risk of transmission. The EVD developed in 692 of 4428 household contacts, a secondary attack rate of 15.6% (95% CI: 14.5-16.7). At least one secondary case occurred in 414 of 1998 households (household transmission rate 20.7%; 95% CI: 18.7-22.3). Of these, 278 households (67.1%) reported one secondary case and 79 (19%) households reported two or more secondary cases. Rates of secondary attack were highest among children under five years of age ($p < .001$), and young children were also more effective transmitters ($p = .01$). Individual risk was not associated with household size. The survey revealed a secondary attack rate of 15.6% and that household transmission occurred in 20.7% of households. A higher attack rate was found among young people, and these latter infected indexes were more likely to transmit the infection. Immediate vaccination around the index case was associated with reduced transmission (p=0.02).

143

DISTRIBUTION OF HISTO-BLOOD GROUP ANTIGEN *FUT2* GENOTYPES AMONG ROTAVIRUS VACCINATED INFANTS IN ZAMBIA

Adriace Chauwa[1], Innocent Mwape[1], Natasha M. Laban[1], Caroline C. Chisenga[1], Michelo Simuyandi[1], Mwelwa Chibuye[1], Obvious N. Chilyabanyama[1], Sody Munsaka[2], Roma Chilengi[1]

[1]*Centre for Infectious Disease Research In Zambia, Lusaka, Zambia,* [2]*The University of Zambia, Lusaka, Zambia*

Rotavirus (RV) is the leading cause of diarrhea among children with the highest burden in developing countries. Despite RV vaccines contributing to the global reduction of RV associated diarrhoea, vaccine efficacy in developing countries remains low compared to developed countries. Histo-blood group antigens (HBGAs) encoded by fucosyltransferase (*FUT)* genes are demonstrated receptors for RV infection. Variation in the secretion of HBGAs conferred by *FUT2* gene polymorphisms is hypothesized to determine susceptibility to RV and influence RV vaccine immunogenicity. We aimed to determine *FUT2* genotype distribution in infants receiving Rotarix® under a randomized controlled trial (RCT) in Zambia and associate this to vaccine immunogenicity. Deoxyribonucleic acid (DNA) was extracted from buffy coat of blood from 90/212 infants enrolled in the RCT and polymerase chain reaction used to amplify *FUT2* gene. G428A polymorphisms of amplified *FUT2* gene were determined by restriction fragment length polymorphisms (RFLP) gel electrophoresis. Secretor genotypes were determined based on size and number of DNA bands from RFLP visualized under UV-transillumination. Association between *FUT2* genotype and gender was tested using chi-square. Enzyme-linked immunosorbent assay will be used to measure RV-IgA titres using serum 4 weeks post-second dose to determine seroconversion, and associations between HGBA status and seroconversion will be tested using multivariate logistic regression. Of 90 infants 47(52.2%) were male and mean (SD) age in weeks 6 (0.6). Distribution of *FUT2* genotypes were 21 (23.3%) AA, 64 (64%) GG and 5 (5.6%) GA corresponding to 69 (76.7%) secretors versus 21 (23.3%) non-secretors. There were no significant differences between; sex and secretor status (p value=0.6). We documented G428A *FUT2* genotypes in Zambian infants with a higher prevalence of the homozygous secretor (GG) genotype, and higher frequency of secretor than non-secretor genotypes. Further evaluation of the association of these *FUT2* genotypes with immune responses to Rotarix® may provide useful insights towards observed variations in vaccine immunogenicity.

145

RISK FACTORS FOR CONTRACTION OF LASSA FEVER VIRUS: A COMPARISON BETWEEN CASES, HOUSEHOLD CONTACTS, AND CONTROLS

Ashley Vellucci[1], Rachel Podgorski[1], Lansana Kanneh[2], Emily Engel[1], Jeff Shaffer[3], Donald Grant[2], John Schieffelin[1]

[1]*Tulane University School of Medicine, New Orleans, LA, United States,* [2]*Kenema Government Hospital, Kenema, Sierra Leone,* [3]*Tulane University School of Public Health, New Orleans, LA, United States*

Lassa Fever (LF) is an acute viral hemorrhagic illness caused by Lassa virus (LASV). Humans are usually infected after exposure to household items contaminated with feces or urine of the *Mastomys* rodent, the reservoir for LASV. Little is known about risk factors of LF in endemic countries, such as Sierra Leone. Kenema Government Hospital's (KGH) Lassa Fever Outreach team conducts case investigations on confirmed LF patients. Data is collected on standardized forms and includes information about housing construction and proximity of the case house to geographic features including garbage pits, farmland, and water sources. Because the *Mastomys* rat is the known reservoir of LF, we also looked at food and water storage within individual households, as well as signs of rodents within households. To identify housing location and construction features associated with increased risk of LF, we compared data collected in the course of these case investigations with data collected from randomly selected houses in Kenema District which has the largest number of confirmed LF cases. Data was available from 191 case investigations. These cases were compared to 403 control households randomly selected from 30 villages, which were randomly selected from Kenema District. Qualitative variables were compared using the Chi Square test. Our analysis revealed that cases were more likely to live within 20m of vegetable plots (48.1% vs 42.4%; p=.0052), were more likely to have rodent holes in their household (87.7% vs 28.8% p<.001), were more likely to have rodent feces in the household (30.9% vs 20.7%; p=.0064), as well as were more likely to have rodent feces in individual rooms within the household (95.2% vs 9.6%; p<.001). Differences were also noted in individual toilet conditions (p<.001), sanitary water sources (p<.0001), and wall construction type (p=.0036). Interestingly, cases were less likely to have water present in the household (94.7% vs 99.07%; p=.0007). Our data highlights risk factors that that may correlate with an increased risk of acquiring LF when comparing confirmed LF case households and control households.

146

OFTEN NEGLECTED VITAL ROAD MAP TO TIMELY AND EFFICIENT MANAGEMENT OF VIRAL DISEASES: NIGERIAN EXPERIENCE

Marycelin M. Baba, Bamidele Soji Oderinde

University of Maiduguri, Maiduguri, Nigeria

Viral diseases have become an increasing concern as the leading cause of morbidity and mortality globally. Effective diagnostic techniques are necessary to identify the etiological agents, mitigate its spread within the community and efficiently manage cases. In Nigeria, routine diagnosis of many viral diseases is lacking except HIV, Polio which is externally sponsored by donors. In the face of epidemics of viral diseases, the limited diagnostic facilities in the country allow these diseases to spread and escalate to complications with high morbidity and mortality rates. Persistent epidemics of Lassa Fever and Yellow Fever have occurred in Nigeria for 51 and 74 years respectively. In April 2019 and 2020, Lassa Fever cases were 537 (122 deaths) and 963 (188 deaths) respectively. Yellow Fever cases in 2017, 2018, 2019 and 2020 ranged from 341 (45 deaths), 159 (26 deaths) 2243 (34 deaths) and 139 (0 death) cases respectively. Other locally undetected viral disease outbreaks include West Nile, dengue, Chikungunya, Zika among others. The atypical signs of these viral diseases with other hyper-endemic diseases like malaria and typhoid and lack of appropriate diagnostic facilities allow viral diseases to be undetected, under-recognized, underestimated and underreported in

the country. Thus viral diseases are often allowed to progress from non -specific (malaria-like phase) to the severe phase of the infection which can enhance spread and impact adversely on treatment/management outcome and mitigation efforts. This review focuses on evaluating and identifying the reasons for lack of routine tests for endemic viral diseases, the impact of such neglect in terms of treatment/management outcome, patients' health, socioeconomic effects on the patients as well as the country.

149

SPATIOTEMPORAL TRANSMISSION DYNAMIC MEASURES WITH SPARSE HEALTH DATA FOR EMERGING DISEASES: AN APPLICATION TO REAL-TIME COVID-19 SURVEILLANCE IN THAILAND

Chawarat Rotejanaprasert

Mahidol University, Bangkok, Thailand

New emerging diseases are public health concerns in which policy makers have to make decisions in the presence of enormous uncertainty. This is an important challenge in terms of emergency preparation requiring the operation of effective surveillance systems. A key concept to investigate the dynamic of infectious diseases is the basic reproduction number. However it can be difficult to apply in real situations due to the underlying theoretical assumptions. In this project we develop a robust and flexible method for estimating disease transmission strength varying in space and time within a hierarchical modeling framework. Due to very limited information when new emerging infections initially appear, it is usual for data to be sparse and it is important, therefore, to account for the overdispersion due to excess zero cases. This has never been considered in the literature because it is difficult to capture that variation in deterministic modeling but could potentially be impactful in the decision making process. A case study is provided of COVID-19 outbreak surveillance in Thailand. This development has potential for broad applicability as an alternative tool for space-time surveillance of emerging diseases.

150

3BASE GENOME APPROACH FOR SCREENING AND DETECTION OF NOVEL MUTANTS OF CORONAVIRUSES

Esther Akiso Omuseni, Faith Sigei, John Waitumbi

USAMRD-A/ KEMRI, KISUMU, Kenya

Coronaviruses belong to a family of *Coronaviridae*, that contains a large number of members that are sub classified as alpha, beta, gamma and delta coronaviruses. Some of the members cause illness in people (e.g., the common cold, SARS, MERS), and others circulate among mammals (e.g., bats, camels, pangolins), and birds. SARS-CoV-2 virus emerged in November 2019 in Wuhan China, and by April 2020, it had infected >2.4 million people globally and killed >160,000. The Covid-19 outbreak is a sad reminder of the pandemic potential of other mutant coronaviruses that lurk in the wild and calls for a paradigm shift in the way we perform diagnostic tests. In this study, a 3 base genome approach will be used to screen and detect known and emerging coronavirus mutants in samples that are being collected as part of Covid-19 virus surveillance in Kenya. The use of 3 base chemistry collapses the 4 base genome to 3 base and will allow detection of any member of the closely-related Coronaviruses (pan-Corona) in the same sample. Therefore, the assay will able to detect all the 7 coronaviruses known to infect human: human coronavirus 0C43; HKU11, HKU13, HK12, SARS-CoV-1, MERS-CoV and SARS-CoV-2, as well as call out for the presence of mutants coronavirus. It's impossible to achieve this with 4 base genome approach. Data that will be accrued as part of the Covid-19 virus surveillance will be updated and discussed during the ASTMH meeting.

151

DETECTION AND CHARACTERIZATION OF A NOVEL STRAIN OF CHAPARE VIRUS DURING AN OUTBREAK OF VIRAL HEMORRHAGIC FEVER IN BOLIVIA, 2019

Maria Morales-Betoulle[1], Roxana Loayza[2], Cinthia Avila[2], Caitlin Cossaboom[1], Sebastian Sasias[2], Mirian Cruz[2], Eliana Gil[2], Gabriela Anez[2], Jimmy Revollo[2], Fernando Morales[2], Carlos E. Alvarez[2], Armando Medina[3], Carla Romero[3], Gricel Alarcon[3], Jhemis Molina[3], Jairo Mendez[4], Shannon Whitmer[1], Ketan Patel[1], John Klena[1], Stuart Nichol[1], Christina Spiropoulou[1], Mary Choi[1], Trevor Shoemaker[1], Joel Montgomery[1]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Centro Nacional de Enfermedades Tropicales, Santa Cruz de la Sierra, Plurinational State of Bolivia,* [3]*Unidad de Epidemiología, Ministerio de Salud, La Paz, Plurinational State of Bolivia,* [4]*Pan American Health Organization, Washington, DC, United States*

Bolivian hemorrhagic fever is caused by Machupo virus (MACV), first isolated and characterized in 1963. MACV has since caused sporadic outbreaks. A novel New World arenavirus, Chapare virus (CHAPV), was identified as the etiology for a fatal case of hemorrhagic fever, known as Chapare hemorrhagic fever (CHHF), in 2004 in Cochabamba. No CHHF cases have been identified since. In June 2019, the Bolivian Epidemiology Services in Caranavi reported three hemorrhagic fever cases of unknown etiology. Specimens from two of the cases tested negative at the Bolivian Center for Tropical Diseases (CENETROP) for MACV, dengue, and yellow fever virus. Because a viral hemorrhagic fever was suspected, specimens were sent to the Viral Special Pathogens Branch, Centers for Disease Control and Prevention (USA) for further testing and identification in the Biosafety Level-4 (BSL4) lab. Using next generation sequencing and virus isolation, we were able to identify and characterize the virus circulating in Caranavi as a strain of CHAPV. We designed primers and probes to develop specific real-time RT-PCR assays for the S and L segments of the virus. In collaboration with the Pan American Health Organization, we deployed those assays to CENETROP and provided training for specimen processing. We detected CHAPV RNA in a variety of human (blood, serum, tracheal aspirates, urine, semen) and rodent specimens associated with the outbreak. Since Machupo and Chapare viruses are BSL4 agents they require special biosafety precautions and inactivation methods during specimen processing. Molecular techniques are preferred when handling those specimens in laboratories that do not have BSL4. Our approach of initial characterization in CDC's BSL4 lab and then deployment of the molecular assays for continued testing in Bolivia has been successful to detect the presence of viral RNA in survivors, identify potential rodent reservoirs, and build local capacity for rapid diagnosis and response to future suspect CHHF cases. This work demonstrates that international partnerships can aid urgent public health responses and highlights the need to enhance laboratory capacity in Bolivia.

152

A NON-INFERIORITY TRIAL COMPARING TWO RECOMBINANT VACCINES (HEPA-B VS. ENGERIX-B) FOR HEPATITIS B AMONG ADULTS IN DHAKA, BANGLADESH

Fahima Chowdhury, Afroza Akter, Tasnuva Ahmed, Taufiqur R. Bhuiyan, Firdausi Qadri

icddr,b, Dhaka, Bangladesh

Worldwide Hepatitis B virus (HBV) is known as one of the imperative causes of mortality and morbidity as well as occupational health hazard among health workers. The burden of HBV infection in Bangladesh is also high, for which the government has introduced hepatitis B vaccination into the Expanded Programme on Immunization (EPI) nationwide since 2009 for new born children. However, HBV vaccination was dependent on imported vaccines as there was no locally manufactured hepatitis B vaccine in Bangladesh. Hence, we conducted a randomized observer blinded non-inferiority clinical trial to assess the immunogenicity and safety of the locally manufactured Hepa-B vaccine in comparison with WHO

prequalified Engerix-B vaccine. Total 158 eligible adult participants were enrolled in this study. Both the vaccines were administered intramuscularly at 0, 1 and 6 months schedule. Baseline and post vaccination anti-HBs titers were measure at different time points. Seroconversion rate post three doses of Hepa-B vaccine was about 99% similar to the comparator Engerix-B vaccine which was 100%. At all time points, the difference in seroconversion rate on anti-HBs titre between Hepa-B and Engerix-B were not significant. The geometric mean test (GMT) ratios of both vaccines at all analysis time points were found >0.5 predefined non-inferiority margin. Soreness at the injection site was the most common symptom for both the vaccines which resolved without any complication. We did not find any serious adverse event throughout the study period. These results suggest that locally manufactured Hepa-B vaccine is non-inferior to the well known licensed Engerix-B vaccine and may precede the licensure in Bangladesh with prospects on easy availability in affordable low price of the hepatitis B vaccine.

153

COMPLICATIONS OF OXIDATIVE STRESS RESPONSES DURING PREGNANCY MALARIA

Rajeshwara Achur[1], Punnath Kishore[1], Valleesha Chandrashekar[1], Kiran Dayanand[1], Srinivas Kakkilaya[2], Poornima Jayadev[3], Suchetha Kumari[4], D. Channe Gowda[5]

[1]*Kuvempu University, Shivamogga, Karnataka, India,* [2]*Light House Polyclinic, Mangaluru, Karnataka, India,* [3]*Sampoorna clinic, Mangaluru, Karnataka, India,* [4]*K.S.Hegde Medical Academy, NITTE (Deemed to be University), Mangaluru, Karnataka, India,* [5]*Pennsylvandia State University College of Medicine, Hershey, PA, United States*

Malaria during pregnancy is a major underestimated global public health problem leading to >10,000 maternal and 100,000 newborn deaths, annually. During pregnancy malaria, parasite sequestration in the placenta leads to leukocyte infiltration and production of inflammatory cytokines and chemokines. Placental parasite sequestration also leads to oxidative stress (OS). To gain better understanding of OS manifestations during placental malaria, this study was conducted in Mangaluru, a south-western coastal city in India, where malaria is highly prevalent. Blood from pregnant women was evaluated for haematological parameters and serum for OS biomarkers such as Malondialdehyde (MDA), Uric acid (UA) and superoxide dismutase (SOD). We also performed histological analysis of infected placental tissue sections for abnormalities and associated complications which were compared with pregnancy outcomes. Correlations were calculated by Pearson's and Spearman rank correlations. Among 105 pregnant women, 34 were healthy controls and infected group comprised of *Plasmodium vivax* (n=48), *P. falciparum* (n=13) and mixed (n=10) malaria. Among 71 infected, 67.6% had mild malaria (MM) whereas 32.4% had severe malaria (SM). The birth weight of infected babies (2.48±0.26 kg) was significantly less than healthy controls (2.64±0.11 kg, P=0.004). The WBC and C-reactive protein levels were increased, whereas haemoglobin, RBC and platelet levels decreased during both malarial infections. The MDA, UA levels increased and SOD levels decreased particularly during *P. falciparum* SM. Histological changes such as syncytial knots, syncytial ruptures and fibrinoid necrosis were observed particularly during *P. falciparum* infections and leukocyte infiltration was observed in *P. vivax* malaria. These altered OS biomarkers are indicators of complications such as severe anaemia, pulmonary oedema, intra uterine growth retardation, premature delivery and low birth weight which occurs both during *P.falciparum* and *P.vivax* malaria. It is of upmost importance to create awareness about preventive measures and antenatal care to reduce pregnancy malaria incidences.

154

UNRAVELING HIDDEN TRANSCRIPTIONAL SIGNATURES FROM *PLASMODIUM FALCIPARUM* FIELD ISOLATES DEPENDENT OF DEVELOPMENTAL STAGE OF CIRCULATING PARASITES

Richard Thomson-Luque[1], Lasse Votborg Novél[1], Joana Martins[2], Nuno M. Sampaio Osório[2], Silvia Portugal[1]

[1]*Centre for Integrative Infectious Diseases, Heidelberg University Hospital, Heidelberg, Germany,* [2]*Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal*

A wide range of clinical outcomes of malaria is often observed in *Plasmodium falciparum* infected patients. Individuals can remain subclinical despite infected, present fever and mild symptoms in uncomplicated malaria cases, or show one or several of the different presentations of severe disease, including the deadliest, cerebral malaria. Increasing parasite exposure and host age associate with the development of protective immunity gradually during childhood, resulting in mostly asymptomatic infections in adults. However, in age-matched individuals with apparently similar parasite exposures, different clinical presentations have been linked to transcriptional differences of *P. falciparum*. Gene expression studies of clinical samples have compared the parasite transcriptional profiles of varying degrees of disease severity, transmission intensity, parasite burden, and different physiological stages of the parasite. Here, we hypothesised that previously published transcriptional signatures attributed to parasites of different clinical presentations may be promoted by varying circulation and hence developmental stages, of infected erythrocytes in the different conditions. We have recently reported that in Mali, subclinical dry season infections present marked transcriptional differences compared to parasites causing malaria in the wet season, but that these differences arise from the longer circulation, even up to the trophozoite stage, of the dry season infected erythrocytes. Revisiting several field studies we interrogate the stage-association of reported DEGs, and we apply bioinformatic tools to determine parasite age-dependent hidden transcriptional signatures. Our new analyses of public datasets and reinterpretation of the results suggests caution should be taken when analysing *in vivo* samples datasets and we propose that different abilities to sequester promote the observed transcriptional differences in circulating *P. falciparum* field isolates and promote different growth rates and associated virulence.

155

ASSOCIATION BETWEEN PLACENTAL MALARIA INFLAMMATORY AND ANGIOGENIC FACTORS IN PREGNANT WOMEN WITH PREECLAMPSIA

Dorotheah Obiri[1], Daniel Oduro[2], Isaac Erskine[3], Jones Amponsah[1], Thomas Addison[1], Kwame Adu-Bonsaffoh[4], Kwadwo Asamoah Kusi[1], Michael Ofori[1], Ben Gyan[1]

[1]*Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana,* [2]*Department of Animal Biology and Conservation Science, University of Ghana, Accra, Ghana,* [3]*Department of Pathology, Korle-Bu Teaching Hospital, Accra, Ghana,* [4]*Department of Obstetrics & Gynecology, Korle-Bu Teaching Hospital, Accra, Ghana*

Placental malaria (PM) and preeclampsia (PE) are associated with inflammatory and angiogenic imbalances in the placenta and maternal circulation resulting in poor pregnancy outcomes. Levels of some inflammatory and angiogenic molecules are increased in conditions of placental dysfunction such as PM and PE. However, it is unknown if the inflammatory and angiogenic pathways during PM precipitate or aggravate the syndrome of PE. A total of 71 pregnant women were recruited: 30 healthy controls and 41 with clinically diagnosed PE. Placental malaria was diagnosed by the histological examination of placental biopsies into active and past parasite infections. Peripheral and placental intervillous plasma samples were obtained at delivery and assessed for markers of inflammation and angiogenesis using a multiplex bead-based assay. Histological analysis of placental biopsies revealed 42 active infections (PE group: n = 27 and non-PE group: n = 15). Past infections were present in

13 placentas (PE group: n = 9 and non-PE: n = 4). Sixteen placentae had no evidence of infection (PE group: n = 5 and non-PE group: n = 11). The placenta showed a higher concentration of inflammatory and angiogenic markers as compared to maternal circulation in both women diagnosed with and without PE. Levels of most proinflammatory markers and anti-angiogenic markers were elevated in maternal circulation plasma samples of PE as compared to non-PE pregnancies. The PE pregnancies with active placental parasites also showed higher levels of proinflammatory and anti-angiogenic markers compared to the non-PE pregnancies. Factors that significantly increased the risk of PE in a multivariate analysis included active parasite infection (AOR = 7.14, 95% CI = 1.1 – 44.7; *P* = 0.04), past infections (AOR = 12.9, 95% CI = 1.1 – 155.5; *P* = 0.04), primigravidity (AOR = 7.2, 95% CI = 1.1 – 48.0; *P* = 0.04) and increased levels of plasminogen activator inhibitor (PAI)-1 molecule AOR = 7.1, 95% CI = 1.3 – 38.3; *P* = 0.02). Our findings show that PE is associated with dysregulated inflammation and angiogenesis which is further enhanced with placental malaria..

156

BLOCKING *PLASMODIUM* HOST CELL INVASION USING SMALL MOLECULE INHIBITORS TARGETING AN ESSENTIAL PROTEIN-PROTEIN INTERACTION

Geervani Daggupati[1], Adam Yasgar[2], Elena Fernandez Alvaro[3], Maria Jesus Almela-Armendariz[3], Maria Isabel Castellote-Alvaro[3], Dolores Jimenez-Alfaro-Mtnez[3], Francisco Javier Gamo[3], Anton Simeonov[2], Louis Miller[4], Prakash Srinivasan[1]

[1]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [2]*National Center for Advancing Translational Sciences, National Institutes of Health, Bethesda, MD, United States,* [3]*Tres Cantos Medicine Development Campus, GlaxoSmithKline, Parque Tecnológico de Madrid, Tres Cantos, Spain,* [4]*Laboratory of Malaria and Vector Research, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States*

The interaction between two parasite proteins apical membrane antigen 1 (AMA1) and its receptor rhoptry neck protein 2 (RON2) is required for *P. falciparum* invasion of RBCs. The conserved interface of this protein-protein interaction lends itself as a potential target for developing parasite-specific antimalarials. We have previously demonstrated that small molecule inhibitors of this interaction exhibit strain-transcending inhibition of merozoite invasion. In this study, using a high throughput AlphaScreen assay, we screened the TCAMS collection of ~13,500 compounds, previously selected for their potent antimalarial activity and drug-like properties, for AMA1-RON2 interaction inhibitors. After an initial single-dose screening we confirmed 63 hits that were positive against both 3D7 and FV0 AMA1, thereby expected to be strain transcendent. We developed a flow-cytometry based invasion inhibition assay using mature synchronized schizonts and identified 24 compounds that exhibited strong inhibition. A standard GIA assay using 3D7 and DD2 strains confirmed all 24 to have potent inhibition. To further determine stage specificity, we conducted a morphological assessment of these compounds. 8 out of 24 did not significantly affect intracellular growth, indicating AMA1-RON2 as the likely primary target. The remaining 11 also inhibited ring/trophozoite, indicating one or more target in these stages in addition to AMA1-RON2. Three compounds that exhibited potent activity in GIA were tested using purified merozoites and confirmed to specifically block parasite invasion. The on-target effects of these inhibitors using various biochemical assays, the potential of these inhibitors to block sporozoite invasion of hepatocytes as well as their potency in combination with current antimalarials will be discussed.

157

HOST BIOMARKERS ARE ASSOCIATED WITH SEVERE MALARIA IN MOZAMBICAN CHILDREN: A MATCHED CASE-CONTROL STUDY

Rosauro Varo[1], Valerie Crowley[2], Antonio Sitoe[3], Lola Madrid[4], Himanshu Gupta[4], Anélsio Cossa[5], Kevin Kain[6], Alfredo Mayor[1], Quique Bassat[1]

[1]*Barcelona Institute for Global Health, Barcelona, Spain,* [2]*S. A. Rotman Laboratories, Sandra Rotman Centre for Global Health, Toronto, ON, Canada,* [3]*Centro de Investigação em Saúde de Manhiça (CISM), Maputo, Mozambique, Manhiça, Mozambique,* [4]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [5]*Centro de Investigação em Saúde de Manhiça (CISM), Manhiça, Mozambique,* [6]*S. A. Rotman Laboratories, Sandra Rotman Centre for Global Health, University Health Network-Toronto General Hospital, Toronto, ON, Canada*

Identifying host biomarkers associated with an increased risk of severe malaria would allow an early diagnosis and better management of this lethal disease. The primary objective of the study was to identify biomarkers of inflammation and endothelial activation differentially expressed in severe versus uncomplicated malaria cases. We conducted a case-control study (2014-2016) in southern Mozambique, with cases being children with severe malaria (SM; defined by World Health Organization criteria) and controls children with uncomplicated malaria (UM) matched by age, sex, and *P. falciparum* parasitaemia. We compared the levels of biomarkers of total parasite biomass (plasma levels of *P. falciparum* histidine-rich protein 2 (HRP-2)) and of host response to infection: Angiopoietin 1 and 2 (Ang-1, Ang-2); Ang-2:Ang-1 ratio, soluble Tie2 (sTie2), brain-derived neurotrophic factor (BDNF), Cystatine C (Cys-C), soluble FMS-like tyrosine kinase-1 (sFt-1), Interleukin (IL-6), Interleukin (IL-8), 10 kDa interferon γ-induced protein (IP-10), soluble tumor necrosis factor receptor 1 (sTNFR-1) and soluble triggering receptor expressed on myeloid cells 1 (sTREM-1). The levels of Ang-2, Ang-2: Ang-1 ratio, sTie-2, sFlt-1, IL-6, IL-8, IP-10, TFNR1, sTrem-1 were significantly higher in children with SM when compared with children with UM (after application of Bonferroni correction for multiple-comparisons, Ang-2, sFlt-1 and IL-8 levels remained significantly increased in children with SM). We also compared levels of biomarkers with The Lambaréné Organ Dysfunction Score (LODS). Levels of IL-6 and IL-8 were higher in children with LODS of 1 when compared to LODS of 0. They were also significantly higher in children with LODS of ≥2. HRP-2 levels were strongly correlated with levels of Ang-2. Host biomarkers associated with endothelial activation and inflammation are able to differentiate between severe and uncomplicated malaria. Ang-2 is the most promising candidate for future clinical applications as it can be used as a triage tool, a marker of response to therapy and a potential therapeutic target for adjunctive interventions for SM

158

BRAIN SWELLING IN PEDIATRIC CEREBRAL MALARIA IS NOT ASSOCIATED WITH ENDOTOXEMIA: HOWEVER, THE GUT MAY PLAY AN IMPORTANT ROLE AS A PARASITE RESERVOIR

Mariko S. Peterson[1], Dumisan Namakhwa[2], Monica Soko[3], Godfrey Mvula[3], Danny A. Milner[4], Terrie E. Taylor[5], Karl B. Seydel[5], Regina Joice Cordy[1]

[1]*Wake Forest University, Winston-Salem, NC, United States,* [2]*Malawi University of Science and Technology, Blantyre, Malawi,* [3]*Blantyre Malaria Project, Blantyre, Malawi,* [4]*Harvard T.H. Chan School of Public Health, Boston, MA, United States,* [5]*Michigan State University, East Lansing, MI, United States*

Cerebral malaria (CM) is associated with endothelial activation and sequestration of mature parasites in the microvasculature of the brain. Increased brain volume is strongly associated with death in young children with *Plasmodium falciparum* infection, but the underlying mechanism is unclear. Sequestration of parasites in the gastrointestinal system has long been observed in autopsy cases and endoscopic biopsy specimens in

individuals with malaria. It has been hypothesized that direct or indirect injury of the epithelial barrier secondary to parasite accumulation may serve as a source for bacterial entry and systemic endothelial activation. We tested the hypothesis that the presence of lipopolysaccharide (LPS) in the blood (endotoxemia) is associated with brain swelling in children with CM. Applying a retrospective analysis to histology and immunohistochemistry data from autopsy cases, we found that parasite accumulation in gastrointestinal tissues was positively associated with parasite accumulation in the brains of children who died with clinically diagnosed CM. In a pilot study using banked plasma collected at the time of admission to hospital from 50 children with CM with and without severe brain swelling, we found that endotoxemia was an uncommon event (5/50). While there were more children with severe swelling who also had endotoxemia, the difference was not statistically significant. LPS concentration was actually slightly negatively correlated with peripheral parasitemia. CM and the associated brain swelling are complex clinical pathologies, with multiple contributory factors including endotoxemia when present. The gut, with its large surface area and high vascularity, may represent an important parasite reservoir that contributes to poor outcomes in malaria. However, our data do not implicate endotoxemia itself as a major contributing factor to increased brain volume in the context of CM.

159

A ROLE FOR *PLASMODIUM FALCIPARUM* CSA LIGAND IN MALARIA PATHOGENESIS BEYOND PLACENTAL SEQUESTRATION

Bethany Jenkins, Patricia Gonzales Hurtado, Robert Morrison, Sachy Orr-Gonzalez, Tarik Ouahes, Lynn Lambert, Michal Fried, Patrick Duffy

National Institutes of Health, Bethesda, MD, United States

Plasmodium falciparum CSA Ligand (PfCSAL), is a conserved protein upregulated in parasites causing placental malaria (PM). We have previously shown that PfCSAL, a peripheral membrane protein, is co-expressed with VAR2CSA, and binds both chondroitin sulfate A (CSA) and VAR2CSA *in vitro*, which suggests a role for PfCSAL in PM pathogenesis. Further, anti-PfCSAL antibody titers are significantly higher in multigravid women and exhibit functional activity, indicating a contribution to PM immunity. PfCSAL is resistant to disruption; out of several lab strains and clinical isolates, viable knockout parasites were only obtained from the CS2 strain, a robust, CSA-binding parasite. We have previously described the CS2 PfCSAL knockout line, which retains both VAR2CSA expression and CSA adhesion in static assays. *In vitro* growth does not differ between PfCSAL null and wildtype CS2 parasites. However, in non-human primate studies, PfCSAL null parasites are attenuated. Here we describe new findings on this attenuated phenotype in *Aotus nancymaae,* including the inability of knockout parasites to infect spleen intact, non-pregnant animals, suggesting PfCSAL facilitates pathogenesis beyond PM. We assess possible mechanisms for this observed fitness cost through comprehensive analyses of the parasite and erythrocyte proteomes, which reveal differences in exported proteins and pathways associated with membrane remodeling. Finally, we present data from ongoing functional assays, including erythrocyte deformability and complement deposition, to further define how PfCSAL contributes to malaria pathogenesis.

161

HEMATOLOGICAL, INFLAMMATORY AND CO-INFECTION EFFECTS OF ASYMPTOMATIC MALARIA INFECTIONS IN ADULTS IN ENDEMIC REGION OF WESTERN KENYA

Edwin Kamau[1], Risper Maisiba[2], Benjamin Opot[2], Rose Adeny[2], Raphael Okoth[2], Farid Abdi[2], Maureen Mwalo[2], Jew Ochola[3], June Otieno[2], Chiaka Nwoga[1], Dennis Juma[2], Valentine Singoei[3], Hosea Akalah[2], John Owuoth[3], Ben Andagalu[2], Amanda Roth[2], Adam Yates[1], Julie Ake[1], Chrisitna Polyak[1]

[1]*U.S. Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [2]*U.S. Army Medical Research Directorate- Africa, Nairobi, Kenya,* [3]*HJF Medical Research International/ U.S. Army Medical Research Directorate – Africa, Kisumu, Kenya*

Asymptomatic malaria infections are highly prevalent in malaria endemic regions, yet the health consequences of these infections are not well understood, especially in adults. The objective of this study was to investigate hematological, inflammatory and co-infection effects of asymptomatic malaria infections in adults enrolled in a prospective, observational cohort study in Kisumu, Kenya. Clinical laboratory tests were performed using standard methods for malaria blood smears and malaria PCR, HIV, CBC/lymphocyte immunophenotyping, chemistries, hepatitis B/C serology, *Schistosoma mansoni* CCA (urine) and HPV PCR. Anemia was defined as Hb<11.0 g/dL. Chi-squared and Fisher's exact tests were used to estimate associations between clinical factors and malaria infections. Data for 575 study participants collected at enrollment between February 2017 and September 2019 were used in the analyses. Parasitemia prevalence was 12.8% by smears, and 32.2% by PCR, and was significantly higher in men ($p<0.0001$), in married individuals ($p=0.01$), low education ($p=0.0002$), and underweight ($p<0.0001$). Parasitemia did not cause anemia. The WBC and platelet counts, and percent neutrophil were significantly higher ($p=0.04$; $p<0.0001$; $p=0.02$ respectively) in aparasitemic individuals. Conversely, monocytes, eosinophil and basophil were significantly higher ($p=0.004$; $p=0.002$; $p=0.003$ respectively) in parasitemic individuals, as well as alanine aminotransferases and creatinine ($p=0.004$; $p=0.006$). Hepatitis C virus, *Chlamydia trachomatis* and *S. mansoni* infections were significantly higher ($p=0.002$; $p=0.05$; $p<0.0001$ respectively) in parasitemic individuals. There were no significant difference in the prevalence of Hepatitis B virus, *Neisseria gonorrhoeae* and HPV infections in parasitemic vs. aparasitemic individuals. Findings in this study clearly demonstrate asymptomatic malaria has health consequences, which are consistent with those seen in symptomatic malaria, and most importantly, these infections seem to play an important role in the prevalence of other co-infections.

162

ASSESSING THE FUNCTIONAL IMPACT OF PFRH5 GENETIC DIVERSITY ON ERYTHROCYTE INVASION PATHWAY UTILIZATION IN *PLASMODIUM FALCIPARUM*

Adam J. Moore[1], Khadidiatou Mangou[2], Fatoumata Diallo[2], Seynabou D. Sene[2], Mariama N. Pouye[2], Bacary Sadio[2], Ousmane Faye[2], Alassane Alassane[2], Amy K. Bei[1]

[1]*Yale School of Public Health, New Haven, CT, United States,* [2]*Institut Pasteur de Dakar, Dakar, Senegal*

Malaria is still responsible for over 405,000 deaths and 228 million cases of disease globally each year, with the large majority of these cases being caused by the *Plasmodium falciparum* parasite subspecies. The RTS,S *P. falciparum* malaria vaccine, the only malaria vaccine ever licensed, is currently being rolled out in three African countries as part of a pilot implementation program. While the creation and licensure of this vaccine are significant accomplishments, the need for a highly effective second generation malaria vaccine remains as important as ever. A major obstacle in the creation of a highly effective vaccine is the extensive genetic diversity displayed in *P. falciparum*. The PfRh5-Basigin ligand-receptor interaction represents an essential step in the merozoite invasion process. Polymorphism in PfRh5 is limited, but in this study, we sought

to investigate 1) the extent of genetic diversity in PfRh5 from a highly endemic region in Senegal, and 2) determine whether specific polymorphic alleles are associated with invasion pathway or immune evasion. In this study, we enrolled *Plasmodium falciparum* consenting patients in Kédougou, Senegal. We performed ex vivo invasion inhibition assays with monoclonal antibodies targeting BSG. Thirty-one ex-vivo invasion assays were performed with field isolates and 95 patient samples, including these 31 isolates, were sequenced at the PfRh5 locus. We are currently analyzing both phenotypic reliance on the PfRh5-BSG invasion pathway and genetic diversity in the PfRh5 locus. This study demonstrates that PfRh5 remains highly conserved and functionally essential, supporting continued development as a blood-stage malaria vaccine target.

163

PLASMODIUM FALCIPARUM MEROZOITE SURFACE PROTEIN 2 FAMILY SPECIFICITY IN CHILDREN WITH OR WITHOUT EARLY RISING ASEXUAL PARASITAEMIA FOLLOWING ARTEMISININ-BASED COMBINATION TREATMENTS

Kazeem Akano[1], Adeyemi Kayode[1], Eregosibina Ayeni[2], Fehintola V. Ajogbasile[1], Onikepe A. Folarin[1], Christian Happi[1], Akintunde Sowunmi[3]

[1]*African Centre of Excellence for Genomics of Infectious Diseases, Redeemer's University, Ede, Nigeria,* [2]*Department of Biological Sciences, Redeemer's University, Ede, Nigeria,* [3]*Department of Pharmacology and Therapeutics, University of Ibadan, Ibadan, Nigeria*

Early rising asexual parasitaemia (ERAP), defined as ≥5% parasitaemia expansion from baseline occurring within 24 hours of therapy, is a phenomenon of artemisinin-based combination treatment. However, there are little evaluations of genetic diversity of parasite populations with parasite expansion phenotype in children with uncomplicated infections. Asexual falciparum parasitaemia was microscopically quantified pre-treatment, 1-2 hourly for 8 hours, and less frequently thereafter for 6 weeks following randomized treatment of acutely malarious children with artesunate-amodiaquine, artemether-lumefantrine or dihydroartemisinin-piperaquine. Baseline characteristics and response to treatment were evaluated in 128 children (64 pairs) with or without ERAP who were matched for age, gender, same day presentation, same baseline parasitaemia and same treatment. Using nested PCR, Block 3 merozoite surface protein 2 (MSP-2) allelic families were assayed in fifty (25 pairs) children. At presentation, proportion of children with temperature >37.4 °C was significantly higher in children with ERAP compared to those without (P=0.04). Geometric mean parasite reduction ratio 2 days post-treatment initiation was significantly higher in children with ERAP compared to those without (P=0.009). Overall, 3D7 clones were significantly more common compared with FC27 clones in this cohort (P=0.0006). Mixed infections occurred in 26%. 3D7 clones were significantly more common compared with FC27 clones (P=0.04) in children with ERAP. Both were similar in children without (P=0.24). In children with ERAP, 3D7, FC27 and mixed infections were similar at presentation and during peak asexual parasitaemia. In conclusion, ERAP children appear to clear parasitaemia slowly but generally, responses to artemisinin-based combination treatments were more uniform in children with and those without ERAP. MSP-2 allelic diversity may not be the only factor for ERAP. Host biology may also be contributory. Mobilization to peripheral circulation following ACT initiation is common to all MSP-2 allelic families.

164

UNVEILING CRUCIAL INTERACTIONS BETWEEN MALARIAL CYSTEINE PROTEASES, FALCIPAINS AND THEIR NATURAL SUBSTRATE AND MACROMOLECULAR INHIBITOR

Rahul Pasupureddy[1], Sonia Verma[1], Akansha Pant[2], Ruby Sharma[3], Sriram Seshadri[4], Ajay Kumar Saxena[5], Rajnikant Dixit[1], Kailash Chand Pandey[1]

[1]*ICMR National Institute of Malaria Research, New Delhi, India,* [2]*Harvard T.H. Chan School of Public Health, Boston, MA, United States,* [3]*Albert Einstein College of Medicine, New York, NY, United States,* [4]*Nirma University, Ahmedabad, India,* [5]*Jawaharlal Nehru University, New Delhi, India*

Malarial proteases constitute an important class of enzymes responsible for various events in parasite life cycle. Cysteine proteases, especially falcipains, are principally involved in the degradation of hemoglobin, the principal source of nutrients for blood stage plasmodium parasites. Our study, through a combination of bioinformatic and mutagenesis analysis, has identified a single amino acid within both falcipains, falcipain-2 (FP2) and falcipain-3 (FP3), crucial for mediating interactions with hemoglobin. This approach is beneficial as the identified residue lies at an exosite protruding away from the active site, thus would likely be less prone to drug pressure. Further, we characterized the interactions between falcipains and their natural macromolecular inhibitor, falstatin. Falstatin, an inhibitor of cysteine proteases (ICP), is unique as compared to its homologues as only a single loop (termed BC loop) is sufficient for the inhibition of falcipains. Our current study suggests that falstatin interacts with FP2 in a multimeric form with ten units of falstatin interacting with ten units of FP2 in a 1:1 stoichiometry, an observation also backed by preliminary bioinformatic analysis. Together, our study aims to characterize falcipain interactions with its natural substrate hemoglobin and the inhibitor falstatin, and attempt to find novel chemotherapeutic targets.

165

NCR3 GENE VARIANTS (-109 C/G AND 132 C/T) INFLUENCE LONGITUDINAL SUSCEPTIBILITY TO MALARIA AND SEVERE MALARIAL ANEMIA IN KENYAN CHILDREN

Clinton O. Onyango[1], Elly O. Munde[2], Samuel B. Anyona[3], Evans Raballah[4], Qiuying Cheng[5], Kristan A. Schneider[6], Christophe G. Lambert[5], Patrick Onyango[7], Douglas J. Perkins[5], Collins Ouma[1]

[1]*Maseno University School of Public Health and Comunity Development, Maseno, Kenya,* [2]*Kirinyaga University School of Health Science, Kerugoya, Kenya,* [3]*Maseno University School of Medicine, Maseno, Kenya,* [4]*Masinde Muliro University of Science and Technology, Kakamega, Kenya,* [5]*Center for Global Health, Department of Internal Medicine, University of New Mexico, Albuquerque, NM, United States,* [6]*Department Applied Computer and Bio Sciences, University of Applied Sciences Mittweida, Mittweida, Germany,* [7]*Maseno University School of Physical and Biological Sciences, Maseno, Kenya*

Plasmodium falciparum malaria is a major cause of pediatric morbidity and mortality in holoendemic transmission areas. The severe clinical outcome in such areas is severe malaria anemia (SMA, Hb<5.0g/dL). Genetic variation in host immune response genes influence susceptibility to SMA. As such, we examined the relationship between variation in the *NCR3* gene, an innate immune receptor, and malaria disease outcomes in Kenyan children (n=1515, aged 3-70 months) at enrollment and over a 36-month follow-up period. Specifically, we examined the association between rs2736191 (-109C/G) and rs11575837 (132C/T), and susceptibility to malaria, SMA, and all-cause mortality in a cohort of children with *P. falciparum* while controlling for anemia covariates. At enrollment, there were no relationships between *NCR3* genotypes/haplotypes and either malaria or SMA. Longitudinally, the genotypes/haplotypes were associated with susceptibility to malaria episodes [132TT genotype (RR=1.556, *P*=0.008)] and SMA [-109CG genotype (RR=1.333, *P*=0.021); GG genotype (RR=1.528, *P*=0.019); C/G haplotype additive effect (RR=1.259, *P*=0.005); CC haplotype additive effect (RR=0.804, *P*=0.006);

GC haplotype (RR=1.334, *P*=0.011) and GC additive effect (RR=1.239, *P*=0.008)], while for SMA episodes [CG genotype (RR=1.358, *P*=0.008); GG genotype (RR=1.462, *P*=0.021); C/G additive effect (RR=1.246, *P*=0.003); CC haplotype (RR=0.806, *P*=0.002); GC haplotype (RR=1.336, *P*=0.006) and GC additive effect (RR=1.218, *P*=0.003)]. Cox regression revealed *NCR3* genotypes/haplotypes to be associated with SMA [CG genotype (HR=1.330, *P*=0.016); GG genotype (HR=1.400, *P*=0.042); C/G additive effect (HR=1.210, *P*=0.013); CT genotype (RR=1.760, *P*=0.016); C/T haplotype additive effect (HR=1.510, *P*=0.022); CC haplotype additive effect (HR=0.830, *P*=0.011); CT haplotype (HR=1.670, *P*=0.021) and GC haplotype (HR=1.290, *P*=0.019)]. None of the *NCR3* variants were associated with all-cause mortality. Our results show variation in *NCR3* influence susceptibility to malaria and SMA during the acquisition of naturally-acquired malarial immunity.

166

USING MAGNETO-OPTICAL DETECTION (MOD) TO TRACK HEMOZOIN LEVELS OVER TIME IN ASYNCHRONOUS CULTURES OF *PLASMODIUM VIVAX*

Brian Grimberg[1], Tyler Witte[2], Cora Layman[2], D'Arbra Blankenship[1]

[1]*Case Western Reserve University, Cleveland, OH, United States,* [2]*Hemex Health, Portland, OR, United States*

This study evaluates the ability of magneto-optical detection (MOD) to detect *Plasmodium vivax* parasites growth throughout a 48-hour period. The MOD device uses a system of lasers and magnets to detect the presence of paramagnetic hemozoin particles that parasite creates as it digests haemoglobin. To perform this test, a sample of frozen Vietnam IV strain of *P. vivax* was thawed, washed (removing all exogenous hemozoin), and diluted to 5000 parasites/uL and 100 parasites/uL using fresh human RBCs which were known to be Duffy Type AA. The cultures were not synchronized and were incubated at 37ºC for 144 hours and sampled every 4 hours. A steady increase in hemozoin content in both the low and high parasitemias was observed over time from the beginning demonstrating the activity of the *P. vivax* parasites even in cultures.

167

THE EPCR-BINDING *PLASMODIUM FALCIPARUM* ERYTHROCYTE PROTEIN 1 (PFEMP1) DOMAINS OF DOMAIN CASSETTE 8 ARE ASSOCIATED WITH ATTENTION DEFICITS IN UGANDAN CHILDREN WITH CEREBRAL MALARIA

Elizabeth Fernander[1], Estela Shabani[2], Dibyadyuti Datta[1], Paul Bangirana[3], Robert Opoka[3], Chandy John[1]

[1]*Indiana University School of Medicine, Indianapolis, IN, United States,* [2]*Harvard University, Boston, MA, United States,* [3]*Makerere University, Kampala, Uganda*

Cerebral malaria (CM) can exhibit lasting neurocognitive deficits in survivors, mostly children in sub-Saharan Africa, but underlying mechanisms are poorly understood. A critical aspect of CM pathogenesis is the binding of PfEMP1, expressed on the surface of infected erythrocytes, to endothelial protein C receptors (EPCR) on brain endothelial cells. Studies have shown increased expression of EPCR-binding domains in children with severe malaria, in particular, the EPCR-binding domains of Domain Cassette 8 (DC8). Here, we investigated whether EPCR-binding domain expression patterns at admission in children with CM are associated with neurocognitive outcomes up to 24 months post-discharge. We used RT-qPCR to measure the expression of CIDRα domains found within DC8 in children with CM and asymptomatic control children. We found associations between the expression of DC8 EPCR binders and parasite density (Spearman's rho, 0.26; p=0.01) and circulating parasite biomass (rho, 0.26; p=0.01). We then examined associations between DC8 EPCR-binding domains expression levels and cognitive outcomes, tested at discharge, 6, 12 and 24 months, using a longitudinal mixed effects model. After adjusting for age, home environment, socioeconomic score, and parasite density, increased expression of DC8 EPCR-binding domains were modestly associated with attention deficits in children ≥5 years with CM (β coef. (95% CI); -0.57 (-1.1, -0.03); p=0.04). We found no other associations with cognitive outcomes over 24 months post-discharge in children ≥5 or children <5 years of age. Further, we did not see associations of other groups of EPCR-binding domains, previously associated with severe malaria. Our findings suggest that increased DC8 expression may be involved in worse outcomes in the area of attention in older children but may not be involved in other pathways leading to neurocognitive impairment in children with CM. Analysis is underway to determine if additional domains, such as DBL domains within DC8, and domains linked with ICAM-1 binding, may be associated with neurocognitive outcomes in CM.

168

THE INFLUENCE OF ASTROCYTE ACTIVATION ON BLOOD BRAIN BARRIER BREAKDOWN DURING EXPERIMENTAL CEREBRAL MALARIA

Franklin Mvo Maloba, Marshall R. Rodel, Thayer K. Darling, Tracey J. Lamb

University of Utah, Salt Lake City, UT, United States

Cerebral malaria is a complication of *Plasmodium falciparum* infection, which most frequently affects young children less than 5 years of age. It can result in seizures, intracerebral hemorrhage, and is often fatal. These symptoms are the effect of the disruption of the blood-brain barrier in response to the localization of *Plasmodium*-infected red blood cells in the neural vasculature. The blood-brain barrier is a complex of cells that tightly regulate the exchange of molecules between the brain and the blood. It consists of endothelial cells that form the vessel walls that are connected by densities of proteins called tight junctions, an extracellular protein scaffolding, and various cell types that interact with the endothelial cells. Astrocytes, a heterogenous population of glial cells in the brain have been shown to be important regulators of the blood-brain barrier. They have been shown to phagocytize infected red blood vessels, and in culture can be activated by extracellular vesicles derived from infected red blood cells. Sequencing of astrocyte RNA in experimental cerebral malaria have confirmed that expression of genes related to activation and immune response is altered in infected mice compared to controls. However, this approach is unable to differentiate responses between different subpopulations of astrocytes. Here, we have used single-cell RNA sequencing to observe different subpopulations of astrocytes that respond differently during ECM malaria in C57BL/6J mice. The characteristics of these subpopulations will be described.

169

HEPATIC DEVELOPMENT OF PURIFIED CRYOPRESERVED *PLASMODIUM FALCIPARUM* SPOROZOITES IN HUMANIZED FRG KO-HUHEP MICE

Michelle Ryan, Alyssa Arnheim, B. Kim Lee Sim, Stephen L. Hoffman, Sumana Chakravarty

Sanaria Inc., Rockville, MD, United States

Sanaria's whole-sporozoite (SPZ) based *Plasmodium falciparum* (Pf) malaria vaccines target the pre-erythrocytic stage of the parasite, developing in the liver. Human trials bypass evaluations of parasite development in this tissue compartment, accessible only through biopsy or imaging techniques. A narrow host-specificity restricted to humans, curtails characterization of PfSPZ in alternate animal models and at the same time Pf inefficiently infect human hepatocytes *in vitro*. Therefore, any assessments of SPZ quality, whether from different strains, or as products of deliberate manipulations have been particularly challenging. For instance, data from mouse infection studies using *Plasmodium yoelii* (Py) SPZ indicate that cryopreservation reduces infectivity of Py SPZ approximately 6- to 10-fold but comparative infection of hepatocytes *in vitro* with fresh and cryopreserved PfSPZ results in non-significant differences. We questioned whether humanized mouse models were better suited to study differential characteristics of fresh vs cryopreserved PfSPZ development in the liver.

Using indirect immunohistochemistry on 6 micron, frozen cryo-sections of infected livers, we detected 25-fold lower numbers of PfLSA-1 positive liver schizonts in mice inoculated with $2.5X10^6$ versus 25-fold less, or $0.1X10^6$ fresh PfSPZ. Compared to $2.5X10^6$ fresh PfSPZ, $1.0X10^6$ cryopreserved PfSPZ resulted in about an 11-fold decline, but was similar to numbers with $0.1X10^6$ fresh PfSPZ. Thus, similar to behavior of Py SPZ in mice, cryopreserved PfSPZ were 4.5-to 10-fold less infective compared to fresh PfSPZ. At the same time qualitative differences were minimal between liver stage parasites that developed from fresh or cryopreserved PfSPZ. Schizonts from fresh and cryopreserved PfSPZ were comparable in size and expressed a multiple parasite markers including PfCSP, PfLSA-1, PfHSP70, and PfMSP1 with similar intensities. The ability to detect quantifiable differences in hepatic infection intensities with PfSPZ inocula differing in strength or cryopreservation status now lends itself to more robust assessments for vaccine development.

170

INVESTIGATION OF MOLECULAR MARKERS OF ANTIMALARIAL RESISTANCE DURING A THERAPEUTIC EFFICACY STUDY CONDUCTED IN MOZAMBIQUE, 2018

Arlindo Chidimatembue[1], Alfredo Mayor[2], Pedro Aide[1], Abel Nhama[1], Lidia Nhamussua[1], Arsenio Nhacolo[1], Quique Bassat[2], Crizoldo Salvador[3], Sónia Enosse[3], Abuchahama Saifodine[4], Eva De Carvalho[5], Baltazar Candrinho[6], Flávio Wate[7], Rose Zulliger[8], Ira Goldman[9], Naomi Lucchi[9], Samaly Souza[9], Eric S. Halsey[10], Venkatachalam Udhayakumar[9], Eusébio Macete[1]

[1]*Manhiça Health Research Center (CISM), Maputo, Mozambique,* [2]*ISGlobal, Barcelona Institute for Global Health, Hospital Clinic - Universitat de Barcelona, Barcelona, Spain,* [3]*National Institute of Health (INS), Ministry of Health, Maputo, Mozambique,* [4]*USAID, Maputo, Mozambique,* [5]*World Health Organization (WHO), Maputo, Mozambique,* [6]*National Malaria Control Program, Ministry of Health, Maputo, Mozambique,* [7]*United States President's Malaria Initiative, US Agency for International Development, Maputo, Mozambique,* [8]*United States President's Malaria Initiative, Centers for Disease Control and Prevention, Maputo, Mozambique,* [9]*Malaria Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States,* [10]*United States President's Malaria Initiative, Centers for Disease Control and Prevention, Atlanta, GA, United States*

Due to the threat of emerging antimalarial resistance, the World Health Organization recommends incorporating surveillance for molecular markers of antimalarial resistance into routine therapeutic efficacy studies (TESs). In 2018, a TES of artemether-lumefantrine and amodiaquine-artesunate was conducted in four sentinel sites in Mozambique: Massinga, Moatize, Mopeia and Montepuez. Mutations in *pfk13, pfcrt* and *pfmdr1*were identified by Sanger sequencing of pre-treatment samples from 109 children. All 109 samples were successfully sequenced for the *pfk13* gene, for the *pfcrt* gene at loci 72-76 and for the *pfmdr1*gene at codons 86, 184, and 1246. No *pfk13* mutations associated with artemisinin resistance were observed. The only *pfcrt* haplotype observed was CVMNK, which is associated with chloroquine sensitivity. For *pfmdr1,* all samples were N86 and D1246 at these two codons, however, polymorphisms were observed at codon 184, with F in 43/109 (39.4%), Y in 42/109 (38.5%) and mixed F/Y in 24/109 (22.0%). In the artemether-lumefantrine arm, there was no association ($p = 0.06$) of 184F with recrudescent samples compared with Day 0 samples from those who would go on to clear infection or experience a re-infection. This polymorphism had a higher prevalence in Massinga (74%) and Moatize (67%) compared with Montepuez (55%) and Mopeia (47%). These findings suggest that artemisinin remains an effective antimalarial in Mozambique and that *Plasmodium falciparum* parasites may have regained sensitivity to chloroquine, but this needs confirmation. The *pfmdr1* 184F polymorphism was highly prevalent and while the extent it can influence resistance is unknown, other studies have found a similar association with increased resistance to lumefantrine. Continued TESs and monitoring of antimalarial resistance markers is warranted in Mozambique for early detection of changes in drug sensitivity.

171

PROFILE OF CENTRAL NERVOUS SYSTEM ADVERSE REACTIONS ASSOCIATED WITH ARTESUNATE AND AMODIAQUINE COMBINATION

Gancia Nsimba[1], Ntamabyaliro Nsengi[1], Jerry Liwono[1], Yves Lula[1], Kahunu Mesia[2], Gaston Tona[2]

[1]*Clinical Pharmacology Unit and pharmacovigilance. University of Kinshasa, Faculty of Medicine, Kinshasa, Democratic Republic of the Congo,* [2]*Clinical Pharmacology Unit and pharmacovigilance. University of Kinshasa, Faculties of Medicine and Pharmaceutical Sciences., Kinshasa, Democratic Republic of the Congo*

ASAQ reported to the National Pharmacovigilance Centre of DRC. Vigilyze was used to search for Nervous system disorders associated with ASAQ reported in DRC from January 2010 to December 2018. ADRs were classified according MedDRA® including the System Organ Class and Preferred Terms. A total of 441 Nervous system disorders ADRs were recorded with 225 (51%) ADRs in female, 216 (49%) ADRs in male; and 55.1% of patient were adult aged between to 18-44 years. Of which: Dizziness (n=301, 68.2%) in which 51.1% of patient were female, and 58.5% of patient were aged between 18 and 44 years. Headache (n=48, 10.9%) in which there is equal numbers of male and female, and 54.1% of patient were aged between 18 and 44years. Somnolence (n=39, 8.8%) in which 53.8% of patient were male, and 38.4% of patient were aged between 18 and 44 years. Dyskinesia (n=10, 2.3%) in which 70% of patient were female, and with equal numbers of patients in the age group between 5 to 11 years and 12 to 17 years. Tremor (n=7, 1.6%) in which 57% of patient were female, and 57% of patient were aged between 18 and 44 years. Extrapyramidal disorders (n=6, 1.4%) in which 60% of patients were male, and with equal numbers of patients in the age group between 12 to 17 years and 18 to 44 years. Seizure (n=5, 1.1%) in which 80% of patient were female, and 80% of patient were aged less then 5 years. Other ADRs were less then 1% of which: Agitation (n=4, 0.9%), Dystonia (n=3, 0.7%), Dysgeusia (n=3, 0.7%), Syncope (n=3, 0.7%), Speech disorder (n=2, 0.4%), Coma (n=2, 0.4%), Tinnitus (n=2, 0.4%), Paraesthesia, Burning sensation mucosal, Loss of consciousness, Disturbance in attention, Altered state of consciousness and eye movement disorder with each (n=1, 0.2%). ASAQ may induce several neurologic disorders, the most frequent of which is dizziness. Most of them seem to occur equally in both sex and all age groups. However, dizziness were more reported in which sex female and patient aged between 18 and 44 years were the most affected.

172

INCREASING PREVALENCE OF MOLECULAR MARKERS FOR RESISTANCE TO ARTEMISININS AND ANTIFOLATE ANTIMALARIALS IN UGANDA

Victor Asua[1], Melissa Conrad[2], Elias Duarte[2], Marvin Duvalsaint[2], Ozkan Aydemir[3], Jennifer Legac[2], Patrick Tumwebaze[1], Roland Cooper[4], Yeka Adoke[1], Samuel Nsobya[1], Jeffrey Bailey[3], Philip J. Rosenthal[2]

[1]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [2]*University of California, San Francisco, San Francisco, CA, United States,* [3]*Brown University, Providence, RI, United States,* [4]*Dominican University of. California, San Rafael, CA, United States*

Progress in malaria control has stalled in much of Africa, and is challenged by drug resistance. In Uganda, artemether-lumefantrine (AL) is recommended for treatment, and monthly sulfadoxine-pyrimethamine is recommended for prevention during pregnancy. To follow resistance trends, relevant genetic polymorphisms were surveyed in *P. falciparum* isolates from patients diagnosed by microscopy or rapid test at 16 sites (50 samples per site per year) in 2018 and 2019. Polymorphisms were characterized by ligase detection reaction fluorescent microsphere assays, molecular inversion probe based next generation sequencing (MIP), Sanger sequencing, and qPCR. Considering transporter polymorphisms associated with resistance to aminoquinolines, prevalences of the PfMDR1

86Y mutation and PfMDR1 amplification were very low (<4% at all sites). Prevalence of the PfCRT 76T mutation has also decreased, but it varied greatly between sites (3-48% in 2018; 6-22% in 2019). Considering K13 propeller domain mutations, the 675V mutation, which has been associated with delayed clearance after artemisinin therapy in Asia and was first identified in Uganda in 2016, had prevalence of 3-10% at 5 northern sites in 2018 and 4-42% at 7 northern sites in 2019. The prevalence of 5 antifolate mutations (PfDHFR 51I, 59R, 108N; PfDHPS 437G, 540E) remained very high; additional mutations associated with high-level resistance had increasing prevalence (by MIP analysis PfDHFR 164L: up to 60% in 2018 and 78% in 2019; PfDHPS 581G: up to 67% in 2018 and 2019). Increased *Plasmepsin* II gene copy number, which is associated with resistance to piperaquine in Asia, was rare. Overall, we found decreased prevalence of transporter mutations associated with aminoquinoline resistance, although the PfCRT 76T mutation retains moderate prevalence at some sites; emergence of a K13 mutation that might mediate delayed parasite clearance in northern Uganda; and increased prevalence of mutations associated with high level antifolate resistance. Continued surveillance for drug resistance markers remains an important priority.

173

ANALYSIS OF *IN VITRO* ACTIVITY AND GENETIC MARKERS OF RESISTANCE OF KENYAN *PLASMODIUM FALCIPARUM* CLONES TO ANTIMALARIALS

Ben Chitama[1], Jesse Gitaka[2], Hassan Hakimi[1], Kesinee Chotivanich[3], Akira Kaneko[4], Kazuhide Yahata[1], Osamu Kaneko[1]

[1]*Nagasaki Institute of Tropical Medicine, Nagasaki, Japan,* [2]*Mount Kenya University, Thika, Kenya,* [3]*Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand,* [4]*Center for Infectious Disease Sciences, Graduate School of Medicine, Osaka City University, Osaka, Japan*

Resistance to antimalarial drugs has posed a challenge in malaria eradication. This study aimed at analyzing both, the *in vitro* activity and genetic markers of resistance of 30 Kenyan clones of *Plasmodium falciparum* collected from Lake Victoria area between 2014-2015 against 8 antimalarial drugs. Results were recorded as concentration of antimalarial drug inhibiting 50% of parasite growth (IC_{50}). Five clones were resistant to chloroquine and all of them showed a CRT triple mutation haplotype (M74I, N75E, K76T). We found a novel mutation V859I in MDR1 potentially associated with increasing IC_{50} of chloroquine resistant clone and is now under further investigation. All clones were resistant to pyrimethamine, and 5 and 1 clones were resistant to mefloquine and lumefantrine, respectively. Remaining clones were susceptible to dihydroartemisinin, piperaquine, amodiaquine, and quinine. Through whole genome sequencing, we found presence of the following genetic markers; DHFR for pyrimethamine (N51I, C59R, S108N / I164L), DHPS for sulfadoxine (A581G, K540E, S436H), and MDR1 for lumefantrine (Y184F). No mutation associated with resistance was seen in *k13*. Periodic surveillance is proposed to understand the ongoing trend of resistance.

174

SAFETY AND EFFICACY OF INTRAVENOUS ARTESUNATE FOR SEVERE MALARIA IN THE UNITED STATES — APRIL 2019 THROUGH MARCH 2020

Francisca Abanyie, Sushama Dhakyl Acharya, Meara Bowe, Kathrine R. Tan

CDC, Atlanta, GA, United States

Of the approximately 2000 cases of malaria diagnosed in the United States annually, 300 are severe and require immediate treatment with intravenous (IV) medications to optimize patient survival. After the discontinuation of IV quinidine, IV artesunate (IVAS) has been the first line medication for the treatment of severe malaria since April 2019, but it is not FDA-approved. CDC is the sole provider of IVAS through its National Artesunate for Severe Malaria Program (NASMP), releasing drug under an Investigational New Drug (IND) mechanism for patients with severe malaria or those with uncomplicated malaria who cannot tolerate oral medication in the United States. Demographic, clinical and laboratory data on patients receiving IVAS are reported to CDC under the IND requirements. To describe the safety and efficacy of IVAS, NASMP data were analyzed. From April 2019 through March 2020, the majority of the 238 patients treated with IVAS were male (59%) and of Black/African American race (77%), median age was 34.3 years (IQR: 14.5-52.4). Almost all [235 (99%)] had severe malaria. At the time of IVAS request, of those with severe malaria: 76% had parasite density ≥5%, 31% had acute kidney injury, and 27% had seizures or impaired consciousness. A total of 224 (94%) patients received all recommended doses of IVAS ; 11 (5%) of which required up to four additional doses. Follow-on oral antimalarial drug was taken by 203 (91%) patients. The median time from first IVAS dose to parasite clearance was 36.2 hours (IQR: 26.5-52.3). Less than 10% (n=22) of patients had an adverse event (AE); 45% (n=10) were serious AEs and were associated with IVAS. The most common AE was post-artemisinin delayed hemolysis, occurring in 8 (36%) patients, 5 (63%) of which required blood transfusions; all recovered. Two patients (0.8%) died due to complications of cerebral malaria. These data indicate that IVAS is a safe and effective drug for the treatment of severe malaria in the United States; parasites were cleared rapidly with very few adverse events. Upon commercial availability, having IVAS in stock at hospitals would provide timely, life-saving treatment to patients with severe malaria.

175

PREVALENCE AND FREQUENCY OF ARTEMISININ RESISTANT *PLASMODIUM FALCIPARUM* IN LAOS DURING 2015-2017

Moritoshi Iwagami[1], Masami Nakatsu[1], Sengdeuane Keomalaphet[2], Phonepadith Khattignavong[2], Pheovaly Soundala[2], Bouasy Hongvanthong[3], Paul Brey[2], Shigeyuki Kano[1]

[1]*Research Institute, National Center for Global Health and Medicine, Tokyo, Japan,* [2]*Institut Pasteur du Laos, Ministry of Health, Vientiane, Lao People's Democratic Republic,* [3]*Center of Malariology, Parasitology and Entomology, Ministry of Health, Laos, Vientiane, Lao People's Democratic Republic*

Lao Ministry of Health adopted a goal to eliminate *Plasmodium falciparum* (*Pf*) malaria by 2025 and all forms of human malaria by 2030. However, artemisinin resistant *P. falciparum* has been reported since 2013 in Laos and is threatening the goal. Mutations of *k13* gene in *Pf* are associated with artemisinin resistance and used as molecular markers for monitoring its resistance. The objective of this study is to assess the distribution and frequency of the *k13* mutations in Laos during 2015-2017 when we collected patients' blood samples from 5 southern provinces and a northernmost province, Phongsaly. In the 5 southern provinces, 2,043 *Pf* samples were analyzed by DNA sequencing. Percentages of the mutations were 56% (660/1,185) in 2015, 45% (179/401) in 2016 and 24% (109/457) in 2017. Predominant mutation was C580Y, which was also predominant in Cambodia, and followed by Y493H, R539T and P574L. The percentage of the mutations was higher in the 2 southernmost provinces. In Phongsaly, all the *Pf* samples (3/3) possessed the C580Y. This study suggested the frequencies of the *k13* mutations seemed to be decreasing in Laos. However, a caution is needed because this tendency might be due to relatively large number of *Pf* samples from Savannakhet with lower percentages of the mutation: 21% (35/166) in 2016 and 5% (14/266) in 2017. In addition, mutation rate as high as 77% (47/61) was still observed in the southernmost province, Champasak, in 2017. Therefore, an intensive surveillance should be continued to monitor artemisinin resistance for its containment to achieve malaria elimination in Laos.

176

EFFICACY OF ARTEMETHER-LUMEFANTRINE AND AMODIAQUINE-ARTESUNATE FOR UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN MOZAMBIQUE, 2018

Abel Nhama[1], Lidia Nhamussua[1], Quique Bassat[2], Eusebio Macete[1], Abuchahama Saifodine[3], Rose Zulliger[4], Flávio Wate[3], Naomi Lucchi Lucchi[5], Samaly Souza[5], Eric Halsey[5], Alfredo Mayor[2], Crizologo Salvador[6], Sonia Enosse[6], Baltazar Candrinho[7], Eva Carvalho[8], Arsenio Nhacolo[1], Arlindo Chidimatembue[1], Pedro Aide[1]

[1]*Centro de Investigação em Saúde de Manhiça, Maputo, Mozambique,* [2]*Institute for Global Health, Barcelona, Spain,* [3]*USAID, Maputo, Mozambique,* [4]*CDC, Maputo, Mozambique,* [5]*CDC, Atlanta, GA, United States,* [6]*Instituto Nacional de Saude, Maputo, Mozambique,* [7]*Programa Nacional de controlo da Malaria, Maputo, Mozambique,* [8]*WHO, Maputo, Mozambique*

Since 2006, artemisinin-based combination therapy has been recommended to treat uncomplicated malaria in Mozambique. Artemether-lumefantrine (AL) and artesunate-amodiaquine (AS-AQ) are first-line treatments. To assess whether AL and AS-AQ remain efficacious, a therapeutic efficacy study was conducted using the 2009 World Health Organization protocol for monitoring antimalarial efficacy. Patients between 6 and 59 months old with uncomplicated *Plasmodium falciparum* malaria (≥ 2,000 - < 200,000 parasites/µl) were assessed from February - September of 2018 in a 28-day *in vivo* efficacy trial in four districts: Montepuez in the north, Mopeia and Moatize in the centre, and Massinga in the south of Mozambique. A Bayesian algorithm was applied to differentiate recrudescence from reinfection using genotyping data of seven neutral microsatellites. A total of 368 and 273 patients were enrolled in the AL and AS-AQ arms, respectively, of whom 9.5% AL (35/368) and 5.1% AS-AQ (14/273) were lost to follow-up. There were 48 AL clinical/parasitological failures and 3 AS-AQ failures. The day 28 uncorrected AL efficacy per site was 72.9% (95% CI 62.2-82.0) in Massinga, 97.5% (95% CI 91.4-99.7) in Moatize, 96.4% (95% CI 89.8-99.2) in Montepuez and 76.2% (95% CI 65.6-84.8) in Mopeia. PCR-corrected AL efficacy was 95.4% (95% CI 87.1-99.0), 100% (95% CI 95.4-100), 100% (95% CI 95.5-100) and 95.5% (87.5-99.2) in Massinga, Montepuez, Moatize and Mopeia, respectively. For AS-AQ, the day 28 uncorrected efficacy was 98.8% (95% CI 93.7-100); 100% (95% CI 95.9-100) and 97.6% (95% CI 91.7-99.7) in Massinga, Montepuez and Mopeia, respectively. The PCR-corrected AS-AQ efficacy was 100% (95% CI 95.8-100), 100% (95% CI 95.9-100) and 98.8% (95% CI 93.5-100) in Massinga, Montepuez and Mopeia, respectively. Both drugs were well tolerated and the frequency of adverse events was less than 2%. This study indicates that both AL and AS-AQ have therapeutic efficacies above the 90% WHO acceptable cut-off. Monitoring of therapeutic efficacy every two years should continue to ensure these treatments remain efficacious.

177

AMPLICON-BASED NEXT GENERATION SEQUENCING OF *PLASMODIUM FALCIPARUM* ANTIMALARIAL RESISTANCE MARKERS

Brooke Clemons, Erica Lasek-Nesselquist, Susan Madison-Antenucci

NYSDOH Wadsworth Center, Albany, NY, United States

Annually ~2,000 malaria cases are diagnosed in the United States and almost all of them are associated with travel. Cases identified in New York State (NYS) alone contribute ~20% of the burden and are primarily caused by *Plasmodium falciparum* (*Pf*). For this species, drug resistance is recognized in all currently available classes of antimalarial drugs. To investigate *Pf* drug resistance in NYS patients we used amplicon-based next generation sequencing (NGS) to detect mutations in six genes that are known to cause, or are strongly associated with, treatment failure. All *Plasmodium* positive samples in NYS are required to be confirmed by the Wadsworth Center Parasitology Laboratory. As a result, we were able to sequence hundreds of whole blood samples with *Pf* from patients who had traveled to over 20 different endemic countries in Africa. The genes targeted include *pfcrt*, *pfdhfr*, *pfdhps, pfmdr, pfcytb* and *pfk13*. These antimalarial resistance markers were detected and compared within and between countries. Data thus far show ~33% of samples have *pfcrt* mutations associated with chloroquine resistance. Approximately 97% of samples have mutations in *pfdhfr* and *pfdhps* which are linked to pyrimethamine/proguanil and sulfadoxine failure, respectively. The data indicate that ~67% of patient samples have mutations in the *pfmdr* gene, reported to cause resistance to multiple drugs including quinine, amodiaquine and artemether-lumefantrine. None of the samples sequenced thus far have contained known resistance mutations in *pfcytb*, therefore overall susceptibility for atovaquone likely remains high. Lastly, we have not observed *pfk13* mutations that are known to cause artemisinin resistance. Using NGS to detect *Pf* drug resistance markers in NYS's population allows for a better understanding of the geographic distribution of antimalarial drug resistance. These data can assist in more accurate prophylaxis recommendations for travelers to endemic countries and can assist clinicians in choosing treatment to reduce the risk of recrudescence once infected.

178

SURVEILLANCE OF EFFICACY AND SAFETY OF ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA IN MAINLAND TANZANIA

Billy E. Ngasala[1], Mercy Chiduo[2], Samwel Bushukatale[1], Twilumba Makene[1], Erasmus Kamugisha[3], Maimuna Ahmed[3], Muhidin Mahende[4], Reginald Kavishe[5], Florida Muro[5], Debora Kajeguka[5], Deus Ishengoma[2], Celine Mandara[6], Ritha Njau[7], Chonge Kitojo[8], Erik Reaves[9], Ssanyu Nyinondi[10], Bilal Kabula[10], Frank Chacky[11], Ally Mohamed[11]

[1]*Department of Parasitology, Muhimbili University of Health and Allied Sciences, Dar es Salaam, United Republic of Tanzania,* [2]*National Institute for Medical Research, Tanga Research Centre, Tanga, United Republic of Tanzania,* [3]*Catholic University of Health and Allied Sciences/Bugando Medical Centre, Mwanza, United Republic of Tanzania,* [4]*Ifakara Health Institute, Dar es Salaam, Dar es Salaam, United Republic of Tanzania,* [5]*Kilimanjaro Christian Medical University College,, Kilimanjaro, United Republic of Tanzania,* [6]*National Institute for Medical Research, Tanga Research Centre,, Tanga, United Republic of Tanzania,* [7]*World Health Organization Country Office, Dar-es-salaam, Tanzania, Dar es Salaam, United Republic of Tanzania,* [8]*PMI-USAID, Dar es Salaam, United Republic of Tanzania,* [9]*CDC, Dar es Salaam, United Republic of Tanzania,* [10]*RTI International, Dar es Salaam, United Republic of Tanzania,* [11]*National Malaria Control Program, Dodoma, United Republic of Tanzania*

The World Health Organization (WHO) recommends regular surveillance of efficacy and safety of artemisinin-based combination therapies (ACT) for the treatment of uncomplicated malaria, which are the currently recommended antimalarial drugs. We conducted a prospective one-arm study to assess the efficacy and safety of artemether-lumefantrine (AL), the first line ACT for uncomplicated malaria in Tanzania. Between May and September 2019, children aged 6 months to 10 years with microscopy confirmed uncomplicated *P. falciparum* malaria who met the inclusion criteria were recruited based on the WHO protocol at four outpatient health facilities in Ilemela, Kyela, Masasi, and Igunga districts. Children were treated with AL twice daily for three days and followed-up on days 3, 7, 14, 21, and 28, and on any day of recurrent illness for clinical and parasitological assessments. Blood was also collected on Whatman filter paper on day 0 and at the time of recurrent infection for polymerase chain reaction (PCR) analysis. The primary outcome measure was PCR-corrected adequate clinical and parasitological response (ACPR) on day 28. A total of 628 children were screened; among these, 349 (55.6%) were enrolled, and 343 (98.3%) of these patients completed 28 days of follow-up or attained the treatment outcomes according to the protocol. There was no early treatment failure; late clinical failure occurred in 20 (5.7%) patients, and late parasitological failure occurred in 34 (9.7%) patients. The day 28

PCR-uncorrected ACPR ranged from 73.9% at Ilemela district to 90.9% at Kyela district. PCR-corrected cure rates were high (> 98%) at all four sites. There were 54 (15.5%) recurrent infections, with the majority (72.2%) occurring in Ilemela and Igunga districts located in high malaria burden regions. The drug was well tolerated, and no serious adverse events were reported. The most commonly reported adverse event was cough in 7% of enrolled patients. These findings suggest that AL is still highly efficacious and well tolerated with minimal adverse events in Tanzania. Recurrent infections were more common in districts located in higher malaria prevalent regions.

179

INVESTIGATING HOST AND PARASITE FACTORS SURROUNDING SEASONAL MALARIA CHEMOPREVENTION IN BAMA, BURKINA FASO

Anyirekun Fabrice Some[1], Thomas Bazié[1], Hanna Ehrlich[2], Justin Goodwin[3], Aine Lehane[2], Catherine Neya[1], Zachari Kabre[1], Jean-Marie Ouattara[1], Martina Wade[2], K. Roch Dabire[1], Brian Foy[4], Sunil Parikh[3], Jean-Bosco Ouedraogo[1]

[1]*IRSS-DRO, BOBO-DIOULASSO, Burkina Faso,* [2]*Yale Schools of Public Health and Medicine, Laboratory of Epidemiology and Public Health, New Haven, CT, United States,* [3]*Yale Schools of Public Health and Medicine, Laboratory of Epidemiology and Public Health, New haven, CT, United States,* [4]*Colorado State University, Fort Collins, CO, United States*

Since 2014, SMC with amodiaquine-sulfadoxine-pyrimethamine has been implemented on a large scale during the high malaria transmission season in Burkina Faso. We report in this paper the prevalence of microscopic and submicroscopic malaria infection at the outset and after the first round of SMC in children under five years old in Bama, Burkina Faso, as well as host and parasite factors involved in mediating the efficacy and tolerability of SMC. Two sequential cross-sectional surveys were carried out in the first month of SMC in a rural area in southwest Burkina Faso. Blood smears and dried blood spots were collected from 106 and 93 children under five, respectively, at the start of SMC and again three weeks later. Malaria infection was detected by microscopy and by PCR from dried blood spots. For all children, day 7 plasma concentrations of desethyl-amodiaquine were measured and CYP2C8 genetic variants influencing AQ metabolism were genotyped. Samples were additionally genotyped for *pfcrt* K76T and *pfmdr1* N86Y, molecular markers associated with reduced amodiaquine susceptibility. 2.8% (3/106) of children were positive for *Plasmodium falciparum* infection by microscopy and 13.2% (14/106) by nested PCR within 2 days of SMC administration. Three weeks after SMC administration, in the same households, 4.3% (4/93) of samples were positive by microscopy and 14.0% (13/93) by PCR (p=0.0007). CYP2C8*2, associated with impaired amodiaquine metabolism, was common with an allelic frequency of 17.1% (95%CI=10.0-24.2). Day 7 concentration of DEAQ ranged from 0.48 to 362.80 ng/mL with a median concentration of 56.34 ng/mL. *Pfmdr1* N86 predominated at both time points, whilst a non-significant trend towards a higher prevalence of *pfcrt* 76T was seen at week 3.This study showed a moderate prevalence of low-level malaria parasitemia in children 3 weeks following SMC during the first month of administration. Day 7 concentrations of the active DEAQ metabolite varied widely, likely reflecting variability in adherence and possibly metabolism. Our findings highlight factors that may contribute to the effectiveness of SMC in children in a high transmission setting.

180

A NOVEL QUINOLINE-LIKE ACTION FOR THE HEME-ARTEMISININ ADDUCT METABOLITE

Victoria A. Balta[1], Wenchuan Ma[2], Katy N. Newlin[2], Ognjen Š. Miljanić[2], David J. Sullivan Jr.[1], Peter G. Vekilov[2], Jeffrey D. Rimer[2]

[1]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [2]*University of Houston, Houston, TX, United States*

Artemisinin's primary mechanism of action involves the cleavage of the endoperoxide bridge by Fe(II) heme, resulting in free radical damage which interferes with numerous parasite functions. During activation by heme, a heme-artemisinin covalent adduct (H-ART) with inactivated artemisinin bound to the heme ring meso carbons can be formed. To date, the effect of exogenously applied H-ART on *Plasmodium falciparum* has not been characterized. Here, we show that exogenously applied H-ART and heme-artesunate adduct (H-ARS), synthesized in reducing conditions, purified and validated by mass spectrometry, inhibits both resistant Kelch13 mutant and sensitive *P. falciparum* in the 5 to 50 nM range in traditional 72 h. IC_{50} assays. Additionally, we show after exogenous H-ART and H-ARS pulses of trophozoites, both adducts are detected with hemozoin heme by mass spectrometry. Six hour ring or trophozoite stage pulse assays with 500 nM of H-ART, H-ARS, artemisinin, artesunate or chloroquine demonstrated that resistant Kelch13 mutant parasites had higher survival compared to sensitive NF54 and CamWT parasites in all drug groups. In ring stage pulses with H-ART, C580Y parasites had a survival rate of 70.5% whereas NF54 and CamWT parasites had survival rates of approximately 17%. Similarly, in ring stage pulses with H-ARS, C580Y parasites had a survival rate of 20.6% whereas NF54 and CamWT parasites showed no survival. Additionally, we found that H-ARS inhibits resistant Kelch13 mutant parasites more than artesunate alone in ring stage assays, with a C580Y H-ARS survival rate of 20.6% and an ARS survival rate of 41.8%. As expected, PfCRT-K76T mutant parasites showed greater survival after chloroquine pulse. In trophozoite stage pulse assays, both H-ART and H-ARS demonstrated near complete inhibition in all strains. These results suggest the irreversible addition of the adduct molecule to the hemozoin crystal within the parasite. Given these results, we conclude that H-ART and H-ARS continue to inhibit *P. falciparum* growth after initial endoperoxide bridge activation through a quinoline like mechanism of action by inhibiting hemozoin crystal extension.

181

EFFICACY AND SAFETY OF ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF *PLASMODIUM FALCIPARUM* MALARIA IN BOHICON AND KANDI, REPUBLIC OF BENIN, 2018-2019

Augustin Kpemasse[1], Ramani Saliou[1], Fortuné Dagnon[2], Alexis Sacca Yarou Maye[1], William Houndjo[1], Alexandre Macedo De Oliveira[3], Leah Morearty[3], Cyriaque Dossou Affoukou[1], Aurore Ogouyemi-Hounto[1], Sakariahou Kpanou[1], Alassane Zoulkaneri[1], Blaise Guezo-Mevo[1]

[1]*Programme Notional de Lutte contre le Paludisme, Cotonou, Benin,* [2]*USAID BENIN, Cotonou, Benin,* [3]*CDC, Atlanta, GA, United States*

In 2006, artemether-lumefantrine (AL), an artemisinin-based combination therapy, was introduced as the first-line treatment for uncomplicated *Plasmodium falciparum* malaria in Benin. In accordance with World Health Organization recommendations to monitor the efficacy of antimalarial treatment at regular intervals, we conducted a therapeutic efficacy study with AL for uncomplicated *P. falciparum* malaria in Bohicon and Kandi, Benin, from 2018 to 2019. Febrile patients aged 6 to 59 months with confirmed falciparum monoinfection and asexual parasite density of 2,000–200,000 parasites/µL received twice daily supervised doses of AL for 3 days. We monitored patients clinically and parasitologically on days 1, 2, 3, 7, 14, 21, and 28. We used genes encoding merozoite surface proteins 1 and 2 (*msp1* and *msp2*, respectively) and the glutamate rich protein (*glurp*) to genotype recurrent infections and differentiate them into recrudescences and reinfections. A molecular analysis to detect mutations in the *K13* gene, which is associated with artemisinin resistance, was carried out on day 0 samples. A total of 205 patients were included in the study. In Bohicon, 105 (91.3%; 95% confidence interval [CI]: 84.6–95.8) of the 115 patients had an uncorrected adequate clinical and parasitological response (ACPR) on day 28. In Kandi, 87 (96.7%; 95% CI: 96.6–99.3) of 90 patientshad an uncorrected ACPR on day 28. Genotype-corrected ACPR rates were 96.3% (95% CI: 90.9–99.0) and 96.7% (95% CI: 96.6–99.3) in Bohicon and Kandi, respectively. The percentage of patients with undetectable parasitemia on day 3 was 100% and 98.9% in Bohicon and Kandi, respectively. No mutation in the *K13* gene of *P. falciparum* was observed. AL remains effective for falciparum malaria

in these two sites in Benin. To allow for early detection of antimalarial resistance, it is important to continue monitoring antimalarial efficacy and prevalence of molecular-resistance markers in Benin to guide treatment policies.

182

IMPACT OF *KELCH13* MUTATION ON CLEARANCE AND CURE OF *PLASMODIUM FALCIPARUM* IN ASIAN AND AFRICAN PATIENTS TREATED WITH ARTEMETHER/LUMEFANTRINE

Cornelis Winnips[1], Celine Risterucci[1], Christian Nsanzabana[2], Victoria Beindl[1]

[1]*Novartis Pharma AG, Basel, Switzerland,* [2]*Swiss Tropical and Public Health Institute, Basel, Switzerland*

The emergence of resistance to artemisinin combination therapies (ACTs) poses a major threat to the global efforts of malaria control and eradication. In 2014, single point mutations in the "propeller" region of the *Plasmodium falciparum* kelch protein gene on chromosome 13 (*kelch13*) were found to be associated with delayed parasite clearance upon administration of ACTs. Thus, proactive surveillance of mutations in *kelch13*, their impact on parasite clearance and treatment outcome is essential to monitor ACT efficacy. Here we present the results from uncomplicated *Plasmodium falciparum* malaria patients treated with Coartem® (artemether-lumefantrine) in a recent phase II clinical trial. We assessed the prevalence of the C580Y point mutation in *kelch13,* at baseline and its relationship to parasite clearance and treatment outcome. Twenty-three patients from Africa and four patients from Asia were treated with Coartem® twice a day for 3 days and followed up for 42 days. Three out of 4 patients from Asia exhibited a C580Y mutant parasite at baseline, and none of the patients from Africa. All 27 patients were cured (PCR-corrected adequate clinical and parasitological response at Day 28). Median parasite clearance half-life was 1.7 h (range 1.0 - 3.6 h) in African patients, 5.3 h (range 5.1 - 7.6 h) in the 3 Asian patients exhibiting a C580Y mutant parasite at baseline, and 10.4 h in the single Asian patient with no C580Y mutation. These findings confirm previously reported data suggesting that Coartem® is clinically effective despite the presence of *kelch13* mutant *P. falciparum*. Despite the low sample size, prevalence of K13 C580Y mutation in Asia was associated with longer parasite clearance time. Larger sample size and assessment of other resistance mutations in *kelch13* and other genes may be of additional value.

183

HEME DETOXIFICATION PROTEIN: AN AVENUE FOR MALARIA CONTROL TO ELIMINATION

Shrikant Nema[1], Sri Krishna[1], Archana Tiwari[2], Praveen Kumar Bharti[1]

[1]*ICMR-National Institute of Research in Tribal Health, Jabalpur, India,* [2]*Rajiv Gandhi Proudyogiki Vishwavidhalya, Bhopal, India*

Accurate and prompt diagnosis of malaria is still a challenge to achieve the elimination goal both locally and globally. In the erythrocytic stage, *Plasmodium* species degrades hemoglobin, releases a toxic product heme. The heme detoxification protein (HDP) helps in the conversion of heme to hemozoin crystal using the histidine residues. Understanding the genetic structure of HDP may be important to provide information for the development of diagnostic biomarkers and designing antimalarial drug which specifically targets hemozoin. Therefore, we have investigated the genetic variation of HDP in the field isolates as well as the clone. A total of 160 *P. falciparum* samples were collected from the Jagdalpur district of Chhattisgarh in India. One-third of the samples were randomly selected to study diversity. Genomic DNA was isolated and the complete P.falciparum hdp gene was amplified and sequenced using Sanger sequencing chemistry. The five samples and one 3D7 reference strain was further cloned into pGEMT-Easy vector system I using molecular TA cloning. A total of 240 positive clones were attempted for sequencing of which 133 clone isolates were analyzed successfully. All the field isolates have shown the one non-synonymous mutation at position 91 which changes from phenylalanine to Leucine (F91L) indicates that it is the ancestral amino acid since all isolates have this change (single mutant). In the clone isolates, besides F91L the double mutant was found with 23.3%, the triple mutant was found with 4.6% in the minor allele of the clone isolates. Due to clonal variation in the same isolate, it indicates that more than one copy of allele is present in the chromosome. Moreover, 8.2% of isolates have changed at histidine level and 91.8% of isolates have changed at non-histidine amino acids. However, we did not find any change in the histidine residues which could hinder hemozoin formation. Amino acid changed at histidine level was found significantly less as compared to the non-histidine amino acid. So, histidine residues may not affect the hemozoin formation; hence HDP may be used as a diagnostic biomarker as well as for the antimalarial drug target.

184

EVALUATION OF A NEWLY DEVELOPED AUTOMATED HEMATOLOGY ANALYZER FOR THE DETECTION OF MALARIA PARASITE IN CLINICAL BLOOD SAMPLES

Kanako Komaki-Yasuda[1], Miki Kawaguchi[2], Kinya Uchihashi[2], Norio Ohmagari[3], Shigeyuki Kano[1]

[1]*Department of Tropical Medicine and Malaria, Research Institute, National Center for Global Health and Medicine (NCGM), Tokyo, Japan,* [2]*Sysmex Corporation, Kobe, Japan,* [3]*Disease Control and Prevention Center, NCGM, Tokyo, Japan*

A flowcytometry-based automated hematology analyzer was newly developed by Sysmex Corporation to measure the number of malaria parasite infected red blood cells (MI-RBC#), and the ratio of them (MI-RBC%). The hematology analyzer also provides the information on parasite species by flagging "Malaria?(p.f)", "Malaria?(others)" or "Malaria?(UNC)" meaning unclassified. In fact, this hematology analyzer is capable of providing the information about them in just about 1 minute. In this study, we evaluated the new hematology analyzer using clinical blood samples from imported malaria patients. The blood samples were collected several times from 78 suspected malaria patients who visited the NCGM hospital from 2017 to Jan. 2019. Among them, 32 patients were diagnosed as "MI-RBC Positive" and the other 46 were diagnosed as "MI-RBC Negative" by the hematology analyzer. These results almost completely matched the PCR diagnosis: 33 patients were diagnosed as positive (1 was *Plasmodium knowlesi*, 2 were *P. vivax*, and the other 30 were *P. falciparum* of which 1 with *P. ovale* and 1 with *P. malariae* infection) and the other 45 were negative. The correlation coefficient between ratios of parasitized erythrocytes determined by the hematology analyzer and microscopy was higher than 0.99. Regarding the discrimination of parasite species between *P. falciparum* and others, the hematology analyzer showed a high coincidence rate of 0.938 as compared with microscopic observation. Six malaria patients were followed by daily measuring of their blood by the hematology analyzer and microscopy until they cleared parasites. The transition of their parasitemia by MI-RBC% and microscopy were well concordant with each other. In conclusion, the information on the MI-RBC#, MI-RBC% and the malaria parasite species that could be rapidly obtained by the newly developed hematology analyzer was proved very accurate and will be useful for supporting clinical malaria diagnosis.

185

IMPLICATIONS OF PROVIDERS' CAPACITY AND FACILITIES' READINESS FOR RELIABLE DIAGNOSIS AND TREATMENT OF MALARIA: A CROSS SECTIONAL SURVEY OF PUBLIC HEALTH FACILITIES IN ETHIOPIA

Bereket H. Alemayehu[1], Mekonnen T. Geleta[2], Abaya Mulugeta[2], Feleke Belachew[2], Negash Seyoum[2], Samuel Girma[3], Gunawardena Dissanayake[3], Hiwot Teka[3], Zenebe Yirsaw[2]

[1]*Columbia University-ICAP, New York, NY, United States,* [2]*Columbia University-ICAP, Addis Ababa, Ethiopia,* [3]*U.S. President's Malaria Initiative, Addis Ababa, Ethiopia*

Quality malaria diagnosis and treatment services require optimal providers' knowledge, skills and practices and well-resourced facilities. A cross-sectional survey was conducted to assess the gaps in laboratory & clinical providers' performances and facilities' readiness to diagnose & treat malaria in 479 public health facilities in the Amhara, Benishangul-Gumuz, Tigray, Gambella, Oromia & Afar regions of Ethiopia between August and December 2018. Data was collected using a pretested, standardized tools that employ index scoring through interviews, direct observation, & document review. Facilities' readiness for laboratory diagnosis & treatment of malaria was assessed by the availability of standard equipment, drugs, supplies, reagents, & job aids. The overall facilities' mean index score of readiness to provide resources for clinical & laboratory services was 39.2% [range: 6.5%- 63.1%] & 42% [range: 2.07% - 100%], respectively. Laboratorians' performances were measured against the 12 laboratory quality management system essentials & clinicians' performances were measured across the continuum of malaria treatment (assess, diagnose, treat and counsel) at all service outlets, respectively. Laboratory average providers' performance score towards the provision of quality malaria diagnostic services was 37.3%, [range: 2.29% - 94.29%]. Clinical providers' performance score of adherence to recommended approaches to assess and diagnose fever cases management was 40.25% [range: 4.03% - 86.7%], & adherence to national guidelines to treat & counsel on malaria cases was 24.7%, [range: 0% - 100%]. Despite the significant expansion of diagnostic & treatment facilities in malaria-endemic settings, gaps in both essential resources & providers' performances in malaria diagnosis & treatment are disconcerting. Lack of adherence to standards of practices in both diagnosis and treatment needs to be addressed through refresher training & on-job mentorship in addition to timely provision of needed resources in order to achieve universal access to quality malaria diagnosis & treatment services.

186

MALARIA QUALITY IMPROVEMENT PROCESSES FOR STRENGTHENED DIAGNOSTIC CAPACITY IN ETHIOPIA

Mekonnen T. Geleta[1], Bereket H. Alemayehu[2], Feleke Belachew[1], Abaya Mulugeta[1], Negash Seyoum[1], Samuel Girma[3], Gunawardena Dissanayake[3], Hiwot Teka[3], Zenebe Yirsaw[1]

[1]*Columbia University-ICAP, Addis Ababa, Ethiopia,* [2]*Columbia University-ICAP, New York, NY, United States,* [3]*U.S. President's Malaria Initiative, Addis Ababa, Ethiopia*

Accurate, early diagnosis and prompt treatment of malaria is a key strategy to defeat malaria. Microscopy is a gold standard diagnostic tool for routine patient management in Ethiopia. However, correct diagnosis has been challenging in areas where the burden of the disease is very high. We developed and implemented malaria-specific quality improvement processes (QIP) for improving malaria diagnostic capacity and system strengthening in 176 USAID/PMI-supported public health facilities in the Amhara, Benishangul-Gumuz, Tigray, Gambella, Oromia and Afar regions of Ethiopia. A prospective assessment was conducted in these health facilities from August 2018- February 2020 to observe the improvement of the quality diagnosis of malaria at health facilities before and after the provision of tailored quarterly technical and logistic support. We measured health facilities' performances in 12 essential elements of quality management systems (QMS) at baseline and at second and third visits using a structured tool. Overall health facilities' performance was dramatically increased from 41% at baseline to 55 % at second, and 65% at third assessment respectively. Of the 176 health facilities, 85 and 23 were assessed for the fourth and fifth time after continued support with an average score of 70 % and 78%, respectively. Of the 12 QSE, process improvement, a single core element of malaria microscopy measurement which accounts for a quarter of all overall score. The score improved from 40% at baseline to 74.4% at the third assessment. Training and regular mentorship coupled with the provision of laboratory supplies and related documents has significantly improved the quality of malaria diagnosis and general laboratory capacity to provide standard services in the PMI supported health facilities.

188

EVALUATION OF DIFFERENT DIAGNOSTIC TECHNIQUES FOR THE DETECTION OF ASYMPTOMATIC MALARIA INFECTIONS IN ARMED FORCES OF LIBERIA PERSONNEL

Hanan El Mohammady[1], Isabelle Nakhla[1], Fatorma Bolay[2], Joseph Kowo[3], Josiah George[3], **Rania AbdelKhalek Nada**[1]

[1]*US. Naval Medical Research Unit-3, Cairo, Egypt,* [2]*Liberian Institute for Biomedical Research, Monrovia, Liberia,* [3]*Armed Forces of Liberia, Monrovia, Liberia*

Successful malaria control and containment strategies demand the implementation of precise diagnostic tools for a more accurate identification of asymptomatic carriers. In endemic malaria countries, most of the asymptomatic cases are not targeted by the national intervention strategies, even though these cases may contribute to parasite endemicity in the country. We compared qPCR, microscopy and rapid diagnostic testing (RDT; BinaxNOW) for the detection of asymptomatic malaria cases. A repeated point-prevalence survey was performed on asymptomatic subjects at Edward Binyan Kessely military barracks, Armed Forces of Liberia . The first survey was in March 2014 with 518 enrollees, whereas the second was in September 2015 including 557 enrollees. Of the total 1075 participants, the three diagnostic tools showed concordant results for 705 (65.6%) individuals, where 32 (3%) were positive for malaria parasite and 673 (62.6%) were negative. The qPCR testing showed higher parasite detection rate (153; 14.2%) compared to microscopy (71; 6.6%) and RDT (51; 4.7%). Of the 153 qPCR positive samples for the genus *Plasmodium*, a species was determined in 96 (62.7%) individuals. *Plasmodium falciparum* was the predominant species identified (90; 58.8%), followed by *P. ovale* (4; 2.6%) and *P. malariae* (2; 1.3%). Apart from the samples providing definite results, a set of collected samples showed borderline results for qPCR and uncertain microscopy reading. In qPCR, 233 (21.7%) samples were equivocal (crossing point, 40-45 cycles), of which, 20 (8.6%) and 7(3%) were positive for microscopy and RDT, respectively. Likewise, microscopic testing showed 31 (2.9%) uncertain readings by two microscopy experts; of these samples 12 (38.7%) and 3 (9.7%) were positive by qPCR and RDT, respectively. In conclusion, our data demonstrates that a significant proportion of asymptomatic individuals have malaria. As well, data shows higher detection rate of malaria parasites in asymptomatic individuals using qPCR, indicating that the use of microscopy and RDT alone for diagnosis of malaria should be revisited for effective malaria control and elimination planning.

189

MAGNETO-OPTICAL DIAGNOSIS OF SYMPTOMATIC MALARIA IN PAPUA NEW GUINEA

Leandra Arndt[1], Agnes Orban[2], Tamarah Koleala[3], Clemencia Ibam[3], Lincoln Timinao[3], Lina Lorry[3], Adam Butykai[2], Peter Kaman[3], Petra Molnar[2], Elma Nate[3], Istvan Kucsera[4], Erica Orosz[4], Brioni Moore[5], Leanne J. Robinson[6], Moses Laman[3], Istvan Kezsmarki[7], **Stephan Karl**[8]

[1]*University of Technoloy, Dresden, Germany,* [2]*University of Technology and Economics, Budapest, Hungary,* [3]*Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea,* [4]*National Public Health*

Center, Budapest, Hungary, [5]Curtin University, Bentley, Australia, [6]Burnet Institute, Melbourne, Australia, [7]Augsburg University, Augsburg, Germany, [8]Australian Institute of Tropical Health and Medicine, James Cook University, Cairns, Australia

Improved methods for malaria diagnosis are urgently needed. In this study, we tested a novel rotating-crystal magneto-optical diagnosis (RMOD) in 964 suspected malaria patients in Papua New Guinea. Capillary blood samples were also subjected, to rapid diagnostic tests, expert light microscopy and polymerase chain reaction to systematically evaluate the capability of RMOD to detect infections. Compared to microscopy, RMOD exhibited an 82 % sensitivity and 84 % specificity to detect any malaria infection. This increased to 87% sensitivity and 88 % specificity for *Plasmodium vivax,* indicating that RMOD could be useful in *P. vivax* dominated elimination settings. Parasite density correlated well with the quantitative magneto-optical signal. RMOD measurements are obtained within minutes and at very low cost. Importantly, residual hemozoin present in malaria negative patients impacted the performance of RMOD. RMOD ability to detect previous infections through measurement of residual hemozoin could be exploited to detect transmission hotspots in low-transmission settings.

192

INTEGRITY OF *PLASMODIUM FALCIPARUM* RAPID DIAGNOSTIC TESTS IN THE CONTEXT OF EMERGING HRP2/3 GENE DELETIONS- A CALL FOR ACTION

Asmerom T. Gebreyohans, Esayas Berhane

Bahirdar University, Bahirdar, Ethiopia

Introduction of rapid diagnostics tests (RDTs) has dramatically increased access to community-level parasitological diagnosis of malaria in Ethiopia since 2007. Both *Plasmodium falciparum* (Pf) and *P. vivax* (Pv) are the prevalent *Plasmodium* species and the multispecies RDTs are used in settings where microscopy is not feasible. Pf is the predominant malaria parasite in the high malaria-burdened north-western region of Ethiopia and recent reports of high incidence of Pf parasites with histidine-rich protein 2 and 3 (pfhrp2/3) genes deletions in bordering countries creates concerns about the sensitivity of the RDTs in use. We have conducted a cross-sectional survey to determine the CareStart™ Malaria HRP2/pLDH(Pf/Pv) Combo RDT's (CS-RDT) diagnostic accuracy for detection of Pf infection using polymerase-chain reaction (PCR) as a reference. During the major transmission season of 2019 (October-December), paired CS-RDT and Pf-specific PCR tests were done on blood specimens collected from 208 malaria suspected adults at two health centers in western Amhara region. The prevalence of Pf malaria confirmed by CS-RDT and PCR were 19.71% (41/208) and 25.96% (54/208), respectively. No Pv was diagnosed with the CS-RDT. The sensitivity and specificity of the CS-RDT were 74.1% (95% Confidence Interval [CI]: 59.2-81.6) and 99.35% (CI: 97.62-100), respectively. The positive predictive value (PPV) and negative predictive value (NPV) of the CS-RDT were 97.56% (CI: 94.13-100) and 81.62% (CI: 76.2-88.43), respectively. The low sensitivity and NPV of the MSpRDT in use are suggestive of possible spread of HPR2/3 gene delated Pf in the area. Confirmation of the presence and estimating prevalence of the gene deletion using serological (antigen) and nested PCR tests should be considered in order to ensure integrity of implemented parasitological diagnosis of malaria.

193

PRECISION DELIVERY OF MALARIA SERVICES IN THE COVID-19 CONTEXT; REMOTE CONFIGURATION AND DEPLOYMENT OF THE REVEAL GEOSPATIAL PLATFORM TO SUPPORT THE DISTRIBUTION OF SEASONAL MALARIA CHEMOPREVENTION IN SOKOTO STATE, NIGERIA

Christina Riley[1], Taiwo Ibinaiye[2], Rachel Murphy[3], Charlotte Ward[3], Shiwan Haruna Dlakwa[2], Frazer Bwalya[1], Ernest Mulenga[1], Livashan Soobramoney[1], Kyle Hutchinson[1], Abimbola Phillips[2], Sol Richardson[3], Kevin Baker[3], Christian Rassi[3], Olatunde Adesoro[2], Anna Winters[1]

[1]*Akros, Lusaka, Zambia,* [2]*Malaria Consortium, Abuja, Nigeria,* [3]*Malaria Consortium, London, United Kingdom*

Although the COVID-19 pandemic has halted a vast number of global health programs, the WHO has urged countries to ensure the continuity of life-saving malaria services in a safe and quality-assured way, including seasonal malaria chemoprevention (SMC). SMC is implemented in four monthly cycles during peak malaria transmission to children under five. Reveal, formerly mSpray, is an open-source platform that uses spatial intelligence to drive delivery of life-saving interventions with precision through identification of the true denominator, real-time coverage and performance tracking, and targeted follow-up of individuals. In 2020, Akros and Malaria Consortium (MC) will pilot the Reveal platform to plan, guide and track delivery of door-to-door SMC to children between 3 and 59 months in two rural health facility catchments (HFCs), one accessible and one hard-to-reach, in Goronyo local government area (LGA), Sokoto State, Nigeria. Reveal will track household-level drug delivery coverage, drug wastage rates, CDD adherence to safety measures and proper hygiene protocols. Under the COVID-19 context and with teams spread across Nigeria, Zambia, and the United Kingdom, Akros and MC conducted a remote country program assessment, validation workshop, tool development, configuration and user testing. The workshop included a comprehensive introduction and demo of the Reveal platform to allow the MC to visualize and validate SMC workflow and data flow translation to achieve optimum in-field alignment. Utilizing satellite imagery and GIS tools, the Akros team in Zambia conducted remote enumeration of two LGAs and guided the MC Nigeria team to map all HFCs within these LGAs. Lessons learned around remote strategies for platform scoping, boundary mapping, configuration and field testing in the 2020 SMC campaign in Sokoto State will be shared, and will also highlight impact on technical capacity building and skills transfer. Initial findings around remote tool configuration and deployment success in the context of COVID-19 will be discussed and can be further explored in the context of program quality assurance, sustainability and remote scale up.

194

DIAGNOSTIC UTILITY OF SALIVA AND URINE SAMPLES FROM FEBRILE PATIENTS IN MALARIA-ENDEMIC SETTINGS

Seda Yerlikaya[1], Katherine Torres[2], Xavier Martiáñez-Vendrell[3], Christoph R. Grünig[4], Alfredo Mayor[3], Dionicia Gamboa[5], Babacar Faye[6], Xavier Ding[1]

[1]*FIND, Geneva, Switzerland,* [2]*Laboratorio de Malaria, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofia, Universidad Peruana Cayetano Heredia, Lima, Peru,* [3]*ISGlobal, Hospital Clínic of Barcelona, Universitat de Barcelona, Carrer Rosselló 153 (CEK building), 08036, Barcelona, Spain,* [4]*Microsynth AG, Schützenstrasse 15, 9436, Balgach, Switzerland,* [5]*Departamento de Ciencias Celulares y Moleculares, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia,, Lima, Peru,* [6]*Service de Parasitologie-Mycologie, Faculté de Médecine, Pharmacie et Odontologie, Université Cheikh Anta Diop de Dakar, Dakar, Senegal*

Malaria rapid diagnostic tests, developed more than 20 years ago, are affordable and easy-to-use lateral flow assays that can detect *Plasmodium* antigens in low volume of capillary blood. While blood collection is generally perceived as minimally invasive, it remains a procedure associated

with potential risk of infection. Moreover, considerable reluctance of patients to provide a blood sample due to cultural, religious, or personal reasons might impact the effectiveness of these tests. The use of bodily fluids that can be collected via non-invasive sampling methods, such as urine or saliva, might overcome these limitations. In this study, we investigated the feasibility of using saliva and urine samples for malaria diagnosis using molecular and immunoassay-based methods. Matching whole blood, saliva, and urine samples were collected from 143 febrile patients with confirmed malaria status in Iquitos, Peru (N=86) and Kedougou, Senegal (N=57). *Plasmodium* DNA in whole blood, saliva, and urine samples was detected using a nested-polymerase chain reaction (nPCR). *Parasitaemia* in whole blood was estimated by expert microscopy and real-time quantitative PCR. The concentration of the malarial antigens, *P. falciparum* Histidine Rich Protein 2 (HRP2) and *Plasmodium* LDH (pLDH), in microscopy-positive samples were measured using an established immunoassay based on xMAP technology (Luminex). Positivity by nPCR in blood, saliva, and urine samples were of 78.3%, 42.5%, and 34.8%, respectively. Amongst microscopy-positive samples, the percentage of nPCR-positive saliva and urine samples increased with blood parasitaemia. For patients with parasitaemia higher than 10,000 parasites/µl of blood, the sensitivity of nPCR on saliva and urine samples was 85.7% and 100%, respectively (N=7). HRP2 and pLDH were detected in 33 and 86 out of 87 microscopy-positive blood samples respectively, but in none of the associated saliva and urine samples. The study shows that while *Plasmodium* DNA can be readily detected in saliva and urine samples, these sample types offer limited potential for tests based on commonly used diagnostic biomarkers of malaria.

195

NEW DIAGNOSTIC DEMONSTRATES IMPROVED LIMIT OF DETECTION FOR *PLSMODIUM VIVAX* IN MULTI-SITE TESTING

Kingkan Pidtana[1], **Gisely Cardoso Melo**[2], Thay Khengheng[3], Worachet Kuntawunginn[1], Rebeca Linhares Abreu Netto[2], Yanka Evellyn Alves Rodrigues Salazar[2], Lek Dysoley[3], Victor Irungu Mwangi[2], Anne Rocheleau[4], Tyler Witte[4], Wuelton Marcelo Monteiro[2], Vanderson de Souza Sampaio[2], Fernando Fonseca de Almeida e Val[2], Kavitha Saravu[5], Marcus Vinicius Guimarães Lacerda[2], Marius Wojnarski[1]

[1]*US Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand,* [2]*Fundação de Medicina Tropical Dr Heitor Vieira Dourado, Manaus, Brazil,* [3]*National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia,* [4]*Hemex Health, Portland, OR, United States,* [5]*Kasturba Medical College, Manipal, India*

Malaria is an infectious disease that led to approximately 435,000 deaths worldwide in 2017. Of the five *Plasmodium* species known to infect humans, *P. falciparum* is the deadliest. However, *P. vivax,* responsible for considerable morbidity and mortality, is more widespread. Current diagnostic methods are inadequate for accurately diagnosing *P. vivax*. Rapid Diagnostic Tests (RDTs) have poorer sensitivity for *P. vivax* compared with *P. falciparum* infections, particularly at lower parasitemia levels. Microscopy is time-consuming and requires expertise. Gazelle™ is a point of care malaria diagnostic device which employs the principle of Magneto-Optical Detection (MOD) to detect hemozoin in a blood sample for diagnosis of malaria. It is an inexpensive (~$1 per test), easy to use, battery operated device which yields results in about 1 minute per test. A dilution study was completed to compare the *P. vivax* limit of detection (LOD) for the Gazelle malaria test to RDTs. The study was performed with 21 samples from three sites in Cambodia, Brazil and India. Each sample was diluted with iterative 50% dilutions in healthy human blood and tested on the Gazelle platform down to the limit of detection (LOD) on the device. The blood dilutions were also tested with SD BIOLINE Malaria Ag P.f/P.v. RDTs. All 21 samples were tested on the Gazelle platform using 15 µl of blood, and 14 of the 21 were also tested with 30 µl of blood. Using the parasite count determined by microscopy, the parasitemia levels corresponding to each dilution level were calculated. Across the samples tested, the lowest calculated LOD for Gazelle was 6 parasites/ul with 30 µl of blood and 8 parasites/ul with 15 µl of blood. In contrast, the lowest LOD for SD BIOLINE RDT was 64 parasites/µl. The average LOD for Gazelle with 30 µl of blood was 67 parasites/µl, more than 12X improvement over the average LOD for SD BIOLINE RDTs, which was 823 parasites/µl. For Gazelle with 15 µl of blood, the average LOD was 126 parasites/µl, a 6X improvement compared with the LOD for SD BIOLINE RDTs. In conclusion, Gazelle on average with 30 µl of blood detected *P. vivax* malaria in a drop of blood at 12X lower parasitemia than SD BIOLINE RDTs.

196

HIGH PROPORTION OF FAINT TEST LINE AND INVALID TEST RESULTS OF MALARIA RAPID DIAGNOSTIC TESTS IN NIGERIA: AN EXPLORATORY STUDY

Sonachi Ezeiru[1], Diwe Ekweremadu[1], Temitope Ipinmoye[1], Olatayo Abikoye[1], Victor Adebayo[1], Genevieve Eke[2], Chukwudi Uche[2], Ezinne Onuba[1], Bala Audu[3], Wellington A. Oyibo[4]

[1]*Catholic Relief Services (CRS)(Principal Recipient of the Global Fund, Malaria in Nigeria), Federal Capital Territory, Abuja, Nigeria,* [2]*Independent Consultant, Abuja, Nigeria,* [3]*National Malaria Control Programme, Federal Ministry of Health, Abuja, Nigeria,* [4]*ANDI Centre of Excellence for Malaria Diagnosis, College of Medicine of the University of Lagos, Lagos, Nigeria*

Malaria rapid diagnostic test (RDT) is one of the tools for parasitological confirmation of malaria recommended by the World Health Organization (WHO) and National Malaria Programme. Over the years, efforts were directed to the quality assurance of RDTs in several rounds of Product and Lot Testing to provide procurement information for countries to select suitable RDTs. Current best practice requires post-market/ pre and post deployment assessments of RDTs to ensure continued product performance. The quality of malaria RDTs was assessed on 25 pre-deployment RDT lots withdrawn from the Pharma-Grade National Warehouse in Lagos State between August 2018 and March 2019 and in health facilities (HFs) in two states of Nigeria in 2018/2019. The RDTs were withdrawn from 47 and 83 HFs in Kaduna (north) and Delta State (south). RDT evaluation was done at the ANDI Centre of Excellence for Malaria Diagnosis, College of Medicine, University of Lagos, Nigeria - a WHO RDT Lot Verification Facility. All RDTs evaluated passed quality control. The two major brands of RDTs withdrawn from the national warehouse were: SD Bioline Malaria Ag, 20 (80%) and CareStart Malaria Ag, 5(20%). No invalid test was observed. However, SD-Bioline Malaria Ag and Carestart Malaria Ag had high operational challenges - 90% and 80% respectively. These were: faint test lines (56%), red background on the nitrocellulose paper of the RDTs (28%), streaked blood-line (36%), and incomplete clearance of the blood flow (16%). CareStart Malaria Ag had higher numbers of faint test lines (89%) than SD Bioline (50%). Three (12%) of the RDTs had no challenge. The challenges in both brands were similar. In Delta State, of the 89 RDTs withdrawn from HFs, 18 (20%) had invalid tests (range,1- 5 invalids/RDT set); faint test line occurred in 83 RDTs (93%) (range, 1 - 24 faint test lines/RDT set). There was no invalid RDT test in Kaduna State but 40 of the 47 RDTs (85%) had faint lines (range, 1-12/ faint test line/RDT set). High proportion of invalid and faint lines could have implications on the accuracy of RDT test interpretation and treatment decisions made by healthcare providers in malaria case management.

197

ASSESSMENT OF INFRASTRUCTURE AND PRACTICES IN PUBLIC HEALTH FACILITIES FOR EFFECTIVE MALARIA MICROSCOPY SERVICES IN THREE STATES OF NIGERIA

Sonachi Ezeiru[1], Diwe Ekweremadu[1], Temitope Ipinmoye[1], Victor Adebayo[2], Olatayo Abikoye[1], Genevieve Eke[3], Chukwudi Uche[3], Ezinne Onuba[1], Bala Audu[4], Wellington A. Oyibo[5]

[1]*Catholic Relief Services (CRS)(Principal Recipient of the Global Fund, Malaria in Nigeria), Federal Capital Territory, Abuja, Nigeria,* [2]*Catholic Relief Services (CRS)(Principal Recipient of the Global Fund, Malaria in Nigeria), Federal Capital Territory, Lagos, Nigeria,* [3]*Independent Consultant, Abuja,*

Nigeria, [4]National Malaria Control Programme, Federal Ministry of Health, Abuja, Nigeria, [5]ANDI Centre of Excellence for Malaria Diagnosis, College of Medicine of the University of Lagos, Lagos, Nigeria

Malaria remains a public health concern in Nigeria as over 57 million clinical cases are reported annually. However, data from population and clinical studies show decreasing prevalence across the country. This makes quality-assured parasitological confirmation of malaria critical in the implementation of the malaria testing and treatment policy. Malaria test results will likely be negative in a number of settings and transmission seasons. In addition, threats of false negative tests with HRP-2-based RDTs due to HRP-2 deletions will need the laboratory. We assessed malaria microscopy infrastructure and practices in three Global Fund-supported states in 132 laboratories. Forty percent (40%) of the building was suitable for malaria microscopy work. Benches for microscopy varied across laboratories: 41(37.3%) had 1-2 benches - an indicator of the low scale of tests that could be done and 47% were adequate. In a sub-set of 126 laboratories, 64.3% do not routinely prepare thin and thick malaria blood films; 81(64.3%) of microscopes in the HFs were not functional; electricity supply was irregular in 60.50% of the laboratories while 70.1% had back-up generators. Availability of quality materials and accessories are important for the performance of microscopy. There were deficiencies that may not enable the laboratories perform accurate and reliable testing - lack of: microscope cleaning fluid (90%), lens tissue (72%), cleaning wipes (81%), immersion oil (29%); standard operating procedures (SOPs) (63.6%), suitable staining racks (62%), reference slides (89%), wash bottles for staining slides (59%), microscope maintenance sheets (92%) and other materials that enhances and assures the desired quality of testing. Recommended Giemsa stain for microscopy is used in 34% of the laboratories while 44% of microscopists have received any kind of training, while 86% of these had received the recommended 10-day basic microscopy training. Laboratory assessment visits in the last 12 months happened in 32% of the laboratories. Commodities and capacity building for microscopy are indicators for measuring access to testing. This gap should be addressed.

198

TRANSMISSION BLOCKING ACTIVITIES OF CIPARGAMIN AND GANAPLACIDE IN ARTEMISININ RESISTANT *PLASMODIUM FALCIPARUM*

Achaporn Yipsirimetee[1], Pornpawee Chiewpoo[2], Rupam Tripura[2], Nicholas P. Day[2], Arjen Dondorp[2], Sasithon Pukrittayakamee[1], Nicholas J. White[2], Kesinee Chotivanich[1]

[1]*Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, [2]Mahidol-Oxford Tropical Medicine Research Unit (MORU), Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand*

Artemisinin resistance in *Plasmodium falciparum* has emerged and spread widely in the Greater Mekong Subregion. New antimalarial drugs are needed urgently for malaria treatment. Cipargamin (KAE609) and Ganaplacide (KAF156) represent novel classes of antimalarial drugs currently in extended phase 2 and phase 3 studies. Both compounds have potent asexual blood stage activities, inhibit *P. falciparum* gametocytogenesis and also reduce oocyst development. In this study, we investigated the effects of cipargamin, ganaplacide and artesunate on male and female gametocytes of artemisinin resistant *P. falciparum* isolates (N=8, K13 mutation; C580Y, G449A and R539T) from Thailand and Cambodia. Parasites were cultured and gametocyte production was induced. The effects on male and female mature stage V gametocytes were assessed by the *P. falciparum* Dual Gamete Formation Assay (PfDGFA). Ganaplacide had the highest potency among the drugs tested. Male gametocytes were significantly more sensitive than female gametocytes ($p<0.001$). The mean (95%CI) IC50 of ganaplacide against male and female gametocytes were 7.2 (6.5 - 7.9) nM and 46.3 (40.7 - 52.9) nM, respectively. Cipargamin had similar potencies against male and female gametocyte, mean (95%CI) IC50 was 102.9 (90.8 - 116.9) nM for male gametocytes and 113.0 (102.9 - 124.3) nM for female gametocytes. Artesunate had no effect against male and female mature stage V gametocytes at 1 uM. Both ganaplacide and cipargamin have significant transmission blocking activities against artemisinin resistant *P. falciparum*.

199

ANTIPLASMODIAL ACTIVITY, ACUTE ORAL TOXICITY AND HEMOLYTIC POWER OF THREE PLANTS FROM CÔTE D'IVOIRE

Jeanne Akissi Koffi[1], Dominique Konan Tano[1], Dieudonne Kigbafori Silue[2], Tresor Marius Dable[1], William Yavo[1]

[1]*Malaria Research and Control Centre of the National Institute of Public Health of Côte d'Ivoire, ABIDJAN, Côte D'Ivoire, [2]Swiss Centre for Scientific Research, ABIDJAN, Côte D'Ivoire*

Despite the efforts made in drug discovery and the development of therapeutic combination for the treatment of malaria, *Plasmodium falciparum* is constantly adapting and developing resistance. This explains the ongoing search for new antimalarial drugs. The objective of this work was to study the antiplasmodial and toxicological activities of three plants traditionally used for the treatment of malaria in Côte d'Ivoire. *Cochlospermum planchonii*'s leaves, *Harungana madagascariensis*'s and *Pericopsis laxiflora* 's barks have been collected, dried and sprayed. Extracts were prepared using various solvents. Thirty-two *Plasmodium falciparum* isolates collected from patients and the NF54 and K1 strains were used for this study. *Ex vivo* and *in vitro* activity tests were performed according to the Rieckmann microtest recommended by the WHO. The spectro-fluorometric technique using SYBR-Green was used to measure the effect of the extracts on the growth of the parasite. The extracts were used on rats for acute toxicity. The hemolysis test was performed on human red blood cells. *Cochlospermum placonii*'s leaves and *Pericopsis laxiflora*'s barks extracts have shown a very good antiplasmodial activity ($1.65 \leq CI_{50} \leq 14.02$µg/ml) and it was noted that the extracts did not present an immediate toxicity by oral administration to 5000 mg/kg. The study of hemolytic activity revealed that the extracts were not hemolytic *in vitro*. *Cochlospermum placonii*'s leaves and *Pericopsis laxiflora*'s barks extracts have an antiplasmodial activity. This study confirms the use of the plants by the traditional healers. We will pursue the work to find the active compounds of the plant for the elimination of malaria.

200

AN INDOLIZIDINE TARGETING *PLASMODIUM* GAMETOCYTOGENESIS AS A NOVEL ANTIMALARIAL AGENT FOR MALARIA TREATMENT AND TRANSMISSION BLOCKING

Jackson M. Muema[1], Joel L. Bargul[2], Meshack A. Obonyo[3], Ramadhan S. Mwakubambanya[3], Merid N. Getahun[4], Redemptah A. Yeda[1], Agnes C. Cheruiyot[1], Hoseah M. Akala[1], Ben Andagalu[1], Jaree L. Johnson[1], Amanda L. Roth[1], James M. Mutunga[1]

[1]*US Army Medical Research Directorate - Africa, Kenya (USAMRD-AIK), KISUMU, Kenya, [2]Jomo Kenyatta University of Agriculture and Technology (JKUAT), Nairobi, Kenya, [3]Egerton University, Egerton, Kenya, [4]International Centre of Insect Physiology and Ecology (icipe), Nairobi, Kenya*

Sexual conversion of *Plasmodium* from a heterogeneous asexual parasite population is regulated by yet-to-be fully characterized switches to sustain malaria transmissions. Yet as an established drug target presenting potential for malaria elimination, few compounds targeting parasite epigenetics, cytosolic protein synthesis and trafficking block gametocytogenesis. Consequent inefficiency of current antimalarials to sufficiently block sexual conversions promotes residual transmissions, warranting novel inhibitors. Exploration of natural resources for potential antimalarials could generate new such inhibitors. Here we explored antimalarial activity of a mesquite agent that exhibits *Plasmodium* gametocytogenesis and transmission blockade as well as asexual growth inhibition. 15 blood samples positive for *Plasmodium species* parasites at ring-stage obtained from individuals presenting uncomplicated malaria were tested for *immediate ex vivo* (IEV) susceptibility to juliprosopine using SYBR Green I assay. Further gametocytogenesis blockade and late-stage (stage IV/V) gametocytocidal analyses were conducted alongside reference

clones. Treatment with juliprosopine potently inhibited *Plasmodium* asexual replication (geometric mean IC_{50} (in µg/mL) 0.531 (95% CI 0.376-0.701) IEV; mean IC_{50} 0.604 ± 0.2101 D6, 1.062 ± 0.1143 F32, 0.378 ± 0.0177 Dd2), arresting trophozoite-to-schizont transition at between 24 and 48 h. Synergism occurred between juliprosopine and standard drugs; dihydroartemisinin, artemisinin, primaquine, piperaquine and desethylamodiaquine. Also, juliprosopine additively interacted with lumefantrine while antagonism was noted with mefloquine, atovaquone, quinine, artemether and chloroquine. Targeting gametocyte-committed rings at either 1×IC_{50} or 5×IC_{50} remarkably arrested stage I-III gametocytes progression, with 100% clearance observed in 5×IC_{50} on day 7 profiling. Also, treatment of stage IV/V gametocytes led to a 90-100% potency up-to 0.0064 µg/mL. Our findings suggest juliprosopine as an amenable scaffold for development of potent malaria transmission-blocking candidates.

201

SECOND GENERATION NOVEL BROAD SPECTRUM ANTIMALARIAL ACRIDONES

Papireddy Kancharla[1], Rozalia Dodean[2], Yuexin Li[2], Brandon Pybus[3], Mara Kreishman-Deitrick[3], Chad Black[3], Qigui Li[3], Raul Olmeda[3], Thu-Lan Luong[3], Heather Gaona[3], Brittney Potter[3], Jason Sousa[3], Sean Marcsisin[3], Diana Caridha[3], Lisa Xie[3], Chau Vuong[3], Qiang Zeng[3], Jing Zhang[3], Ping Zhang[3], Hsiuling Lin[3], Kirk Butler[3], Norma Roncal[3], Lacy Gaynor-Ohnstad[3], Susan Leed[3], Christina Nolan[3], Frida Ceja[4], Stephanie Rasmussen[4], Brett Bayles[4], Roland Cooper[4], Michael Riscoe[2], Jane Kelly[2]

[1]*Portland State University, Portland, OR, United States,* [2]*Portland VA Medical Center, Portland, OR, United States,* [3]*Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [4]*Dominican University of California, San Rafael, CA, United States*

The global impact of malaria remains staggering despite extensive efforts to eradicate the disease. With increasing drug resistance and the absence of a clinically available vaccine, there is an urgent need for novel, affordable, and safe drugs for prevention and treatment of malaria. Previously, we described a novel antimalarial acridone chemotype that is potent against both blood-stage and liver-stage malaria parasites. Here, we present an optimization process that has produced a second-generation acridone series with significant improvements in efficacy, metabolic stability, pharmacokinetics, and safety profiles. These findings highlight the therapeutic potential of dual-stage targeting acridones as novel drug candidates for further preclinical development.

202

ACTIVITY OF SULFATED POLYSSACCHARIDES AGAINST *PLASMODIUM FALCIPARUM* EXPORTED PROTEINS

Jennifer Mumbe Mutisya[1], Victor Mobegi[2], Johnson Kinyua[3], Martha Nginya[1], Edwin Mwakio[1], Gladys Chemwor[1], Raphael Okoth[1], Redemptah Yeda[1], Agnes Cheruiyot[1], Benjamin Opot[1], Dennis Juma[1], Hosea Akala[1], Ben Andagalu[1], Amanda Roth[1]

[1]*United States Army Medical Research Directorate-Africa,The Malaria Drug Resistance Laboratory, Kisumu, Kenya,* [2]*University of Nairobi, Nairobi, Kenya,* [3]*Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya*

The onset of artemisinin resistance in South East Asia calls for research of more drug compounds that have antiplasmodial activity. *Plasmodium falciparum* (Pf) utilizes the process of host erythrocyte remodeling using exported proteins to enhance pathogenesis using PHIST (*Plasmodium*-Helical Interspersed Sub-Telomeric) domain proteins. Identification of these proteins as drug targets heralds the need for discovery of inhibitors that could double up as drug compounds that act by inhibiting these proteins in *Plasmodium falciparum*. The aim of this study is to identify these antimalarials from sulfated polysaccharides. The compounds are then tested for activity against an exported protein PHISTb/RLP1 (PHISTb protein containing Ring infected erythrocyte binding Like protein). 300 samples of ongoing, epidemiology of malaria and drug resistance sensitivity patterns in Kenya study were analyzed by targeted sequencing of PHISTb/RLP1 gene using Sanger Sequencing. The sequenced reads were mapped on the reference Pf3D7 protein sequence of PHISTb/RLP1 using the CLC Main Workbench. Homology modelling of both reference and mutant protein structures was achieved using LOMETs tool. The models were refined using ModRefiner for energy minimization. Ramachandran plot were generated by ProCheck to assess conformation of amino acids in the protein model. Protein binding sites predictions was assed using FT SITE software. We searched for the prospective antimalarials from PubChem. The compounds were screened according to Lipinski's rule of five of a drug compound. Docking experiments were achieved using autodock vina and analysis results visualized in PyMOL. 13 drug compounds with antiplasmodial activity were identified. 10 of the drug compounds interacted with amino acid residues in PHISTb and RESA domains, showing potential activity against these proteins. A comparison of protein-ligand interactions between the reference and those proteins carrying mutations within the functional domains revealed change the binding site locations on such protein sequences. These interactions provide lead compounds for new anti-malarial molecules.

203

I0N VITRO ANTIMALARIAL ACTIVITY AGAINST *PLASMODIUM FALCIPARUM* OF NOVEL 4-AMINOQUINOLINE HYDRAZONES

Rachael Magwaza[1], Muna Abubaker[2], Buthaina Hussein[1], Ilaria Russo[3], Sally Freeman[1], Niroshini Nirmalan[2]

[1]*Division of Pharmacy and Optometry, School of Health Sciences, Faculty of Biology, Medicine & Health, University of Manchester, Manchester, M13 9PT, UK., Manchester, United Kingdom,* [2]*Department of Environment and Life Sciences, University of Salford, Salford, M5 4WT, UK, Salford, United Kingdom,* [3]*Centre for Applied Entomology and Parasitology, School of Medicine & School of Life Sciences, Keele University, Staffordshire ST5 5BG, UK, keele, United Kingdom*

The emergence of resistance to the first-line antimalarial drugs calls for the urgent need to develop new antimalarial agents. Drug repositioning offers an effective alternative to *de novo* drug design to counter the spread of drug-resistant malaria. Here, we report the 4-aminoquinoline hydrazones as novel antimalarial leads. The compounds were originally designed as inhibitors of NRH:quinone oxidoreductase 2 (NQO2), a potential therapeutic target in cancer chemotherapy. *Plasmodium falciparum* contains a functionally similar type II NADH:dehydrogenases known as *Pf*NDH2 and this forms the basis of the rationale for the selection of these leads. The 4-aminoquinoline hydrazones have been identified as potent *in-vitro* inhibitors of the multi-drug resistant strain K1 of *Plasmodium falciparum* with nano-molar IC_{50} values. Time-course assays were carried out to define the activity time-lines of the leads, which indicated that the compounds are fast-acting. The cytotoxicity assay initially indicated a narrow therapeutic index but this was later explained by the changes in drug exposure time when assessing the dose-response on K1 strain. Analyses of stage-specificity revealed that the ring stage of the parasite life cycle was most affected. Drug combination studies showed synergy of the 4-aminoquinoline hydrazones with some known antimalarial drugs.

204

ACYL-COA SYNTHETASE 10 IDENTIFIED AS NOVEL *PLASMODIUM FALCIPARUM* DRUG TARGET

Selina Bopp[1], Charisse Flerida Pasaje[2], Pamela Magistrado-Coxen[1], Robert L. Summers[1], Sumanta Dey[2], Sebastian Smick[2], Armiyaw S. Nasamu[2], Allison R. Demas[1], Victoria Corey[3], Maria De Gracia Gomez-Lorenzo[4], Virginia Franco[4], Angela M. Early[5], Amanda K. Amanda K.[1], Danny Milner[1], Javier Gamo[4], Elizabeth A. Winzeler[3], Sarah K. Volkman[1], Jacquin Niles[2], Dyann F. Wirth[1]

[1]*Harvard T.H. Chan School of Public Health, Boston, MA, United States,* [2]*Massachusetts Institute of Technology, Cambridge, MA, United*

States, [3]University of California, San Diego, La Jolla, CA, United States, [4]GlaxoSmithKline, Tres Cantos, Spain, [5]The Broad Institute, Cambridge, MA, United States

The emergence and spread of drug resistance to current antimalarial therapies remains a pressing concern, escalating the need for compounds that demonstrate novel modes of action and overcome the development of drug-resistance. The Malaria Drug Accelerator (MalDA) consortium has adopted a chemogenomic approach to identify targets of the most promising compounds from chemically diverse libraries. This approach identified the acyl Co-A synthetase (ACS) enzyme family as new potential drug targets, where mutations in ACS10 and ACS11 were identified from selections of two structurally unrelated compounds (MMV665924 and MMV019719). ACSs activate fatty acids (FA) scavenged from the host, which can then be used for protein modification, phospholipid biosynthesis, and FA elongation. By introducing the observed mutations into the 3D7 parental line using CRISPR/Cas9, we demonstrated that the mutations in ACS10 and ACS11 are indeed sufficient to phenocopy the resistance phenotype of the selected lines. We generated conditional knock-down lines and confirmed that ACS10 is essential and the target of the drugs. Knock-down of ACS11 revealed that it is non-essential and therefore our hypothesis is that mutations in ACS11 are conferring resistance but that ACS11 is not the direct target. This is an interesting differentiation between the two ACS proteins whereby ACS10 is targeted by the compounds while mutations in ACS11 are more likely involved in a secondary resistance mechanism.ACS genes are highly polymorphic and surprisingly, the ACS10 M300I mutation identified here was present at 78% in a Malawi parasite population. We obtained Malawian isolates and found that an isolate containing the M300I polymorphism was fivefold more resistant to MMV665924 than a matched ACS10 wild type Malawi isolate. We present ACS10 as a potential new drug target, but natural population variants could reduce efficacy of some compounds inhibiting ACS10.

205

PRIMAQUINE TOXICITY: METABOLIC EFFECTS OF QUINONE METABOLITES GENERATED IN HUMAN ERYTHROCYTES

Kaitlyn Ryan[1], Narayan D. Chaurasiya[1], Washim Khan[2], Yanhong Wang[2], HMT Bandara Herath[2], NP Dhammika Nanayakkara[2], Larry A. Walker[2], Babu L. Tekwani[1]

[1]*Southern Research, Birmingham, AL, United States, [2]University of Mississippi, Oxford, MS, United States*

Primaquine (PQ) has broad therapeutic utility for treatment, prophylaxis, radical cure, and prevention of transmission of malaria. Mass drug administration of PQ has been suggested for clearance of residual gametocytemia in malaria endemic regions for malaria control. However, hemolytic toxicity in individuals with genetic deficiency of glucose 6-phosphate dehydrogenase (G6PD) limits the use of PQ for malaria radical cure and prevents wide-spread use of this drug in public health. Oxidative metabolites generated through CYP-mediated pathways have been implicated in hemolytic toxicity of PQ in G6PDd deficient people. Previous studies in our labs have suggested oxidation of PQ directly in human erythrocytes. Primaquine-5,6-*ortho*quinone (PQoQ) was identified as a major metabolite in human erythrocytes. More recently we have identified another quinone, 6-methoxyquinoline-5,8-*p*-quinone (MQ*p*Q), derived from dealkylation of PQ-quinone-imine and PQoQ and MQ*p*Q were tested for their effects on normal and G6PD deficient human erythrocytes. PQoQ and MQ*p*Q treatments produced dose-dependent methemoglobin accumulation and oxidative stress in normal and G6PD deficient human erythrocytes, which have both been suggested as potential early markers of hemolytic response. The hemolytic response generated by PQ*o*Q was relatively mild compared to the robust response generated by MQ*p*Q. Co-incubation of MQ*p*Q with Cytochrome P_{450}/b_5 reductase did not increase hemolytic response against human erythrocytes while PQoQ treatment required Cytochrome P_{450}/b_5 reductase to generate hemolytic response. Co-treatment with NAD(P)H:quinone oxidoreductase 2 (NQO2) inhibitors namely melatonin, quercetin and resveratrol synergized the hemolytic response of MQpQ. These observations suggest a protective effect from NQO2 against hemolytic toxicity from quinone metabolites. This study provides new insight into the mechanism of hemolytic toxicity of PQ.

206

A WORLD-WIDE DIVERSE FUNGAL LIBRARY FOR DRUG DISCOVERY AGAINST MALARIA TRANSMISSION

Guodong Niu, Xiaohong Wang, **Jun Li**

Florida International University, Miami, FL, United States

Fungal metabolites are sources for new drugs against infectious diseases and cancers. To obtain a library with enough diversity, we collected about 2300 soil samples and 2300 plant samples from 36 areas of four countries in Africa, Asia and North America. The collection areas covered different climate zones of the world. Nearly ten thousand fungi were isolated. Sequences of nuclear ribosomal internal transcribed spacer from randomly selected 40 isolates showed that >80% were different from each other. Using these fungi, the crude fungal extract library was established. We also examined the usability of this fungal library against parasitic malaria transmission, gram positive and negative bacterial pathogens, and leukemia cells. *Penicillium thomii* and *Tolypocladium album* were identified to produce secondary metabolites to block *P. falciparum* transmission to *An. gambiae* and to kill myelogenous leukemia cell line K562 respectively. A set of candidate fungi against bacterial pathogens are also reported. Thus, this fungal library is valuable for drug discovery.

207

TARGET-BASED VIRTUAL SCREENING OF AN FDA-APPROVED DRUG LIBRARY IDENTIFIES AGE-OLD ANTI-MALARIAL DRUGS GENTAMYCIN AND CLINDAMYCIN NOVEL MECHANISM OF ACTION

Yash Gupta, Raman Mathur, Ravi Durvasula, Prakasha Kemapaih

Loyola University Chicao Stritch school of Medicine, Maywood, IL, United States

There has been a decline in Malaria cases globally but factors like Population density, poverty, and encroachments of new areas are maintaining a steady risk of undoing all successful efforts of malaria control. In 2018, an estimated 218M cases were reported worldwide. Sixty-seven percent of deaths were children mostly from sub-Saharan Africa. While, Chemotherapy with antiparasitic drugs is the basis for malaria treatment; the increasing incidence of antimalarial drug resistance to the first-line ACT underpins an urgent need for new antimalarial drugs, ideally with a novel mode of action (MoA). Knowledge of MoA can help aid selection of suitable partner drugs as well as give an insight into the potential for drug resistance. Target-based drug discovery facilitates the rational development of novel therapeutics and alleviates host toxicity at the designer level. In this work, homology model of PfHDAC1 was built using multi-template modeling by the I TASSER server. The model constructed was optimized and simulated for 20ns simulation before virtual screening using the Desmond module (Schrodinger-2020-1). Known inhibitor SAHA was docked at the active site using the Glide module. FDA approved library consisting of ~2900 compounds were obtained from the ZINC database. Virtual screening was performed using a wizard with HTVS, SP, and XP docking and screening stages followed by MM-GBSA shortlisting. Tryptamine, Gentamycin, and Clindamycin were the top hits with Glide energies-49, -47, and -63 respectively. This was surprising as Gentamycin is routinely used to check bacterial contamination in malarial cultures and Clindamycin is a known inhibitor of apicoplast protein translation. To validate this further both complexes were subjected to a 20 ns simulation revealing a very strong interaction. Both Gentamycin and Clindamycin flew off due to big size un-bound bulk. We are currently performing enzymatic assays with PfHDAC1 for inhibition kinetics and in-vitro testing. Our *Plasmodium* specific interaction data can be exploited to derivatize & design novel specific antimalarials.

208

HEAT SHOCK PROTEIN 90 (HSP90) AS A TARGET FOR ANTIMALARIAL DRUG DEVELOPMENT

Amanda K. Lukens[1], Sabine Ottilie[2], Prianka Kumar[2], Andrea Cheung[2], Juan Carlos Rodriguez[2], Madeline R. Luth[2], Nimisha Mittal[2], Krittikorn Kumpornsin[3], Gareth Girling[3], Charisse F. Pasaje[4], Kerstin Gagaring[5], Christopher Mansfield[6], Emmanuelle LeBlanc[7], Luke Whitesell[7], Leah E. Cowen[7], Emily R. Derbyshire[6], Ulf Ribacke[8], Case W. McNamara[5], Jacquin C. Niles[4], Marcus Lee[3], Elizabeth A. Winzeler[2], Dyann F. Wirth[9]

[1]*The Broad Institute, Cambridge, MA, United States,* [2]*University of California San Diego School of Medicine, La Jolla, CA, United States,* [3]*Wellcome Sanger Institute, Hinxton, United Kingdom,* [4]*Massachusetts Institute of Technology, Cambridge, MA, United States,* [5]*Calibr at Scripps Research Institute, La Jolla, CA, United States,* [6]*Duke University School of Medicine, Durham, NC, United States,* [7]*University of Toronto, Toronto, ON, Canada,* [8]*Karolinska Institute, Stockholm, Sweden,* [9]*Harvard T.H. Chan School of Public Health, Boston, MA, United States*

The emergence and spread of drug resistance to frontline antimalarial therapies necessitates the replenishment of the drug development pipeline with new drug targets and compounds that have novel modes of action. Heat shock protein 90 (HSP90) has long been investigated as a drug target for anticancer and anti-infective therapies. The natural product, geldanamycin (GDA), is active against *P. falciparum* HSP90 (PfHSP90) suggesting this target could be exploited for antimalarial drug development. To better understand the mode of action of GDA, we performed *in vitro* evolution experiments and isolated PfHSP90 mutant parasite lines resistant to the compound. The mutations (N9K, D88Y, and G112W) map to the nucleotide binding domain of PfHSP90 and are hypothesized to disrupt inhibitor binding. Recent efforts as part of the Malaria Drug Accelerator identified BMS-983970 from the ReFRAME library as having both liver and asexual blood stage antimalarial activity. BMS-983970 resistant parasites were generated and a mutation identified in PfHSP90. The mutation results in an A41S amino acid change in the nucleotide binding domain and confers ~4-fold resistance to BMS-983970 in whole cell phenotypic assays (Dd2 EC_{50} = 1.6nM; drug selected EC_{50} = 6.4nM). CRISPR-Cas9 genome editing was used to engineer PfHSP90:A41S or D88Y allelic replacement cell lines and validate the role of these resistance mutations. PfHSP90:A41S-edited parasites phenocopy the drug selected lines showing ~7-fold resistance to BMS-983970; efforts to isolate the PfHSP90:D88Y allelic replacement lines are ongoing. Additional *in vitro* selections with BMS-983970 in the model organism *S. cerevisiae* also identified HSP90 mutations that confer resistance to the molecule. Furthermore, BMS-983970 directly inhibited the human and fungal HSP90 enzymes in *in vitro* activity assays. Taken together, these data suggest that BMS-983970 targets HSP90, representing a starting point for further development. Overall, these studies highlight PfHSP90 as a promising antimalarial drug target that warrants further target-based approaches to identify potent selective inhibitors.

209

COMPUTATIONAL ANALYSIS OF PLASMEPSIN V PROTEIN AND PREDICTION OF CRUCIAL RESIDUES

Sonia Verma[1], Akansha Pant[2], Rahul Pasupureddy[1], OP Singh[1], Veena Pande[3], Kailash C. Pandey[1]

[1]*National Institute of Malaria Research, New Delhi, India,* [2]*Harvard T. H. Chan school of public Health, Boston, MA, United States,* [3]*Department of Biotechnology, Kumaun University, Nainitaal, Uttrakhand, India*

Plasmepsin V, a malarial aspartic protease has a crucial role in export of ~350 proteins that have pentameric PEXEL motif. PMV is essential for parasite survival and is a promising antimalarial target in asexual blood stage. Recently solved crystal structure of PMV from *P.vivax* with its PEXEL mimetic inhibitor has revealed how inhibitors docked into the active site and found two unique protein segment (Nap1 insert & flap loop). Further, to explore the importance of PMV Nap 1 insert & flap loop, we have performed bioinformatics studies which includes PMV sequence and structure analysis. The sequence alignment which included PMV sequences of various plasmodium species shows conserved catalytic triad (DTG, DSG), Nap 1 insert and flap loop. The structure analysis of PMV includes MD simulations, protein-ligand interaction, hydrogen bonding, disulphide bond, salt bridge and bfactor analysis. The protein-ligand interaction shows that the catalytic aspartic residues (Asp80, Asp313) interact with ligand via hydrogen bonding and residues from flap loop also interactions with ligand via hydrogen bonding (Cys140, Glu141)or Vander Waal interactions (Gln137, Ser138, Tyr139). These results show that the flap loop may have a role in the proper positioning of the ligand in the active site. The highly conserved Cys117 residue from Nap loop make a disulphide bond with Cys128 and also interact with Gly131 via hydrogen bonding. The residue Cys117 could be responsible for Nap loop stability. A residue Asn127 from Nap loop interact with Glu134 a flap loop residue via a salt bridge. This residue may play important role in the transfer of stimulus from Nap loop to flap loop for direction shift upon protein binding. The bfactor analysis reflects the fluctuation of the atoms about their average position and provides important information about the protein dynamics. The bfactor analysis of PvPMV structure shows that a Nap loop segment (119-NEECPF-124) have high bfactor representing high thermal motion in that segment. This motif due to its high mobility has a role in exporter proteins recognition. These findings may have implication in site-directed mutagenesis studies.

210

CITRULLUS LANATUS (WATERMELON) RIND EXTRACTS AMELIORATED SOME BIOCHEMICAL AND HISTOPATHOLOGICAL CHANGES IN *PLASMODIUM BERGHEI* INFECTED MICE (SWISS STRAIN)

Ehimwenma Sheena Omoregie[1], Nekpen Erhunse[1], Francis Irabor[2], Osamudiamen Ebohon[2], Aishat Mary Momodu[3]

[1]*University of Benin, Benin City, Nigeria,* [2]*Michael and Cecilia Ibru University, Warri, Delta State, Nigeria,* [3]*Ambrose Alli University, Ekpoma, Benin City, Nigeria*

Malaria, a mosquito-borne disease, is a major public health concern in Nigeria with high rate of mortality especially in children below the age of five years and pregnant women. The quests for appropriate vaccines/drugs for the management of this condition still remain elusive. The incidence of resistant strains of *Plasmodium* parasite to available antimalarial drugs is of great concern. This has necessitated the continuous search for new sources of possible antimalarial compounds/drugs. In this study, we evaluated the antiplasmodial property of aqueous and ethanol extracts of *Citrullus lanatus* (watermelon) rind in *Plasmodium berghei* (ANKA strain) infected mice. Water melon fruit rind was selected for this study based on local claims of its antimalarial property. The fruit rind extracts were prepared using standard protocol. Sets of albino mice of the Swiss strain were used for the antiplasmodial activity based on the classical four-day suppressive test against *Plasmodium berghei*. After 2 hr of inoculation, the mice were treated orally with the extracts (250 and 500 mg/kg body weight) or the reference antimalarial drug, chloroquine (10 mg/kg body weight). Treatment was done once daily for 4 days. Results from the study shows that the infected mice treated with the ethanol extract (500 mg/kg) of rind had the highest suppression of the *P. berghei* parasite when compared to the infected control. Percentage suppression was found to increase in a dose - dependent manner. Some changes in hematological indices, liver function parameters, oxidative stress indices and histopathology of the spleen, brain and liver tissues were observed as a result of the *P. berghei* infection in the mice. However, treatment of the infected mice with the different doses of the aqueous and ethanol rind extracts ameliorated these changes, in most cases, positively. The findings from this study support the use of *C. lanatus* rind in ethnomedicinal practices. The *C. lanatus* rind may be useful as part of functional food system in the management of malaria infection.

213

CYTOCHROME P450 2D6 (CYP2D6) ACTIVITY AND TIME OF EXPOSURE TO MALARIA MODULATE THE RISK OF *PLASMODIUM VIVAX* RECURRENCE

Ana Carolina R. Silvino[1], Flora S. Kano[1], Marcelo A. Costa[2], Cor Jesus F. Fontes[3], Irene S. Soares[4], Cristiana F. Brito[1], Luzia H. Carvalho[1], **Tais Nobrega Sousa**[1]

[1]*René Rachou Institute/Fiocruz, Belo Horizonte, Brazil,* [2]*Federal University of Minas Gerais, Belo Horizonte, Brazil,* [3]*Federal University of Mato Grosso, Cuiabá, Brazil,* [4]*University of São Paulo, São Paulo, Brazil*

The relapsing peculiarity of *Plasmodium vivax* is one of the prime reasons for sustained global malaria transmission. Primaquine (PQ) is the only commercially available drug for the treatment of relapsing malaria strains and its efficacy is dependent on metabolic activation by cytochrome P450 2D6 (CYP2D6). Here, we hypothesized that host immune response to malaria parasites modulates susceptibility to *P. vivax* recurrences in association with CYP2D6 metabolism. We performed a 10-year retrospective study by genotyping *CYP2D6* polymorphisms in malaria-exposed individuals treated with chloroquine-PQ therapy from the Brazilian Amazon. Recurrence was defined as a new episode (microscopy-diagnosed) occurred between 29 and 180 days from the initial episode. The number of malaria episodes was obtained from the SIVEP-Malaria database (from 2003 to 2013). Immune response against a panel of *P. vivax* antigens ($PvDBP_{II}$, PvAMA1 and $PvMSP1_{19}$) was evaluated by serological assays in three cross-sectional surveys (2008-2009). In the study area, *P. vivax* recurrences were responsible for approximately 18% of clinical cases reported annually. The prevalence of individuals with reduced CYP2D6 activity was 25% (out of 252). Impaired CYP2D6 activity showed a higher risk of multiple episodes of *P. vivax* recurrence (risk ratio 1.75, 95% CI 1.2-2.6, $P = 0.0035$). An important finding was a reduction of 3% in the risk of recurrence (risk ratio 0.97, 95% CI 0.96-0.98, $P < 0.0001$) per year of malaria exposure, which was observed for individuals with both reduced and normal CYP2D6 activity. Accordingly, subjects with long-term malaria exposure and persistent antibody responses to various antigens showed fewer episodes of malaria recurrence. Our findings have direct implications for malaria control since it was shown that non-immune individuals who do not respond adequately to treatment due to reduced CYP2D6 metabolism may present a significant challenge for sustainable progress towards *P. vivax* malaria elimination.

214

PREVALENCE OF *PLASMODIUM FALCIPARUM* INFECTION IN PREGNANT WOMEN OF SOUTHERN MOZAMBIQUE

Gloria G. Matambisso[1], Pau Cistero[2], Gizela Bambo[3], Henriques Mbeve[1], Judice Miguel[1], Cardoso Melembe[1], Sonia Maculuve[1], Clara Menéndez[4], Clara Menéndez[5], Eusébio Macete[1], Alfredo Mayor[6]

[1]*Manhica Health Research Center, Manhica District, Mozambique,* [2]*SGlobal, Barcelona Ctr. Int. Health Res. (CRESIB), Hospital Clínic - Universitat de Barcelona, Barcelona, Spain,* [3]*Manhica Health Research Center, Manhica Disctirct, Mozambique,* [4]*Manhica Health Research Center and ISGlobal, Barcelona Ctr. Int. Health Res. (CRESIB), Hospital Clínic - Universitat de Barcelona, Barcelona, Spain,* [5]*2ISGlobal, Barcelona Ctr. Int. Health Res. (CRESIB), Hospital Clínic - Universitat de Barcelona, Barcelona, Spain,* [6]*Manhica Health Research Center (CISM) and 2ISGlobal, Barcelona Ctr. Int. Health Res. (CRESIB), Hospital Clínic - Universitat de Barcelona, Barcelona, Spain*

Increases in malaria-related harmful effects observed among Mozambican pregnant women after drastic malaria declines during the last decade suggest that close monitoring of the transmission is needed to quickly identify rebounds in adverse outcomes, especially in areas embarking on malaria elimination. We conducted a three-year *prospective observational* study (from 2016 to 2019) at three health centers with different levels of malaria transmission in Maputo Province (Manhiça District Hospital, Ilha Josina and Magude Health Centers). Finger-prick blood samples onto filter papers collected at first antenatal visit and delivery, are being analyzed by real-time quantitative polymerase chain reaction (qPCR) to assess the presence of *Plasmodium falciparum* (*Pf*). A total of 1423 women were recruited in Ilha Josina Health Centre; 10419 in Manhica District Hospital and 5794 in Magude Health Center. In parallel, cross-sectional surveys conducted in May 2017-2019, estimated *Pf* infection prevalence by rapid diagnostic test in an age-stratified simple random sample of the population of Manhiça and Magude districts. The prevalence of HIV infection in pregnant women is 32.1 % in Manhiça; 24.9 % in Ilha Josina and 26.9 % in Magude. Among these,11132 samples were analyzed by qPCR. The prevalence of *Pf* infection per site is 5.07% (298/5876) in Manhiça; 3.87% (158/4081) in Magude and 20.42% (240/1175) in Ilha Josina. There was a correlation between *Pf* infection prevalence among pregnant women attending the first antenatal visit and *Pf* infection prevalence in children 2-10 years , with a Pearson r of 0.9994(p=0.0006). At the first antenatal visit, the prevalence of maternal anemia (Hb < 11 g/dl) was 56,9% in Manhiça; 42,5% in Magude and 46,2% in Ilha josina. At maternity visits, the prevalence of maternal anemia was 60.7% in Manhiça; 38,4% in Magude and 21,9% in Ilha Josina. We are completing the analysis to asses which is the impact of the *Pf* infection on maternal anemia and child weight at birth. This preliminary analysis suggests a strong correlation between *Pf* prevalence detected in children 2-10 years from the community and in pregnant women.

215

INTEGRATION OF MALARIA CASE BASED SURVEILLANCE (COCONUT) AND DHIS2 IN ZANZIBAR TO IMPROVE DATA USE IN DECISION MAKING

Joseph J. Joseph[1], Humphrey Mkali[1], Shabbir Lalji[1], Abdul-wahid Al-mafazy[1], Ssanyu S. Nyinondi[1], Bilali Kabula[1], Abdullah S. Ali[2], Faiza B. Abbas[2], Mohamed H. Ali[2], Wahida S. Hassan[2], Ahlam Saeed[3], Chonge Kitojo[4], Naomi Serbantez[4], Bisanzio Donal[5], Richard Reithinger[5], Jeremiah Ngondi[5], Mike McKay[5]

[1]*RTI International, Dar es salaam, United Republic of Tanzania,* [2]*Zanzibar Malaria Elimination Programme, Ministry of Health,, Zanzibar, United Republic of Tanzania,* [3]*Health Management Information System,Ministry of Health, Zanzibar, United Republic of Tanzania,* [4]*US President's Malaria Initiative, United States Agency for International Development, Dar es salaam, United Republic of Tanzania,* [5]*RTI International, Washington DC, DC, United States*

Surveillance is a core intervention in supporting malaria elimination through optimal monitoring and response of malaria interventions. DHIS2 data flow model improves access to information and timeliness from the point of data entry. It further allows greater functionality for reportingand analyzing aggregate reports. The Ministry of Health (MoH) began using DHIS 2 in 2006. It was reporting malaria indicators on monthly basis at district level. However, the Zanzibar Malaria Elimination Program (ZAMEP) needed weekly data to monitor malaria cases and trends. In 2008, a health facility based Malaria Epidemic Early Detection System (MEEDS) was established. Case Based Surveillance (*Coconut*) was further established in 2012 to track notified cases up to household level. *Coconut* and DHIS2 allow interoperability and integration of malaria data. We supported ZAMEP to streamline the various malaria systems and data sources through data integration. Data elements from *Coconut* were integrated to DHIS2 by using an application programming interface (API). We integrated four data elements: malaria cases- by type of detection; by age; by gender and case classification. The integrated data is synchronized daily. To enhance the data feedback loop to improve data uptake, use, and quality, we built a dashboard using DHIS2 modules to visualize the data. DHIS2 dashboard is simple-to-use, and provides quick updates on performance of programmatic indicators through tables, charts and maps, aggregated by administrational divisions (Shehia/ward, district, and region). Integration goes in line with World Health Organization's (WHO) recommendation of utilizing a single system primarily owned by the MoH (DHIS 2 in our case), and centralizing data collation and management for informed decision making.

216

THE EFFECT OF IRON DOSE DURING PREGNANCY ON RISK OF MALARIA CARRIAGE AND GAMETOCYTEMIA

Sarah Boudova, Methodius Tuuli

Indiana University, Indianapolis, IN, United States

Malaria and anemia are two major threats to pregnancies around the globe. Current recommendations are that all pregnant women receive supplementation with iron-folate. However there is concern that iron supplementation may actually increase risk of malaria. Iron-deficiency anemia has been associated with protection from malaria in pregnant women, and iron supplementation has been shown to reverse these protective effects *in vitro*. A large meta-analysis of existing studies showed no increased risk of malaria in women supplemented with oral iron. However, supplementation practices vary widely and the effect of iron dose on risk of malaria has not been examined. Additionally, most studies have focused on clinical malaria and maternal-fetal outcomes. Gametocytes are the transmissible form of the malaria parasite, and it has previously been demonstrated the pregnant women are a reservoir of submicroscopic parasitemia and gametocytemia. In vitro data show a critical role for iron in gametocytogenesis. Therefore, it is plausible that iron supplementation in pregnancy may increase malaria transmission through increased gametocyte carriage. We are conducting a cross sectional study in Accra, Ghana correlating iron supplementation with parasitemia and gametocytemia at the time of presentation for labor and delivery. Korlebu Teaching Hospital has over 10, 000 deliveries per year. We anticipate including 3000 patients in this study. We are collecting detailed demographic and prenatal information including dose of iron supplementation. The primary outcome is parasitemia and gametocytemia diagnosed from maternal blood samples using real time reverse transcription PCR. We will compare rate and density of parasitemia and gametocytemia across doses of iron supplementation. Multivariable logistic regression will be used to control for confounders. As malaria control programs move toward eradication it has become critical to examine not only the health impacts of malaria control measures, but also the effect on malaria transmission. This study will provide essential information for malaria elimination programs.

217

SEASONAL *PLASMODIUM FALCIPARUM* GAMETOCYTE PATTERNS IN WESTERN KENYA

Colins O. Oduma[1], Sidney Ogolla[1], Harrysone Atieli[2], Ming-Chieh Lee[3], Andrew K. Githeko[4], John Githure[4], Arlene Dent[5], James W. Kazura[5], Guiyun Yan[3], Cristian Koepfli[6]

[1]*Kenya Medical Research Institute, Kisumu, Kenya,* [2]*3. School of Public Health, Maseno University, Maseno, Kenya, Kisumu, Kenya,* [3]*Program in Public Health, College of Health Sciences, University of California, Irvine, CA, United States,* [4]*4. International Center of Excellence for Malaria Research, Homabay, Kenya,* [5]*5. Case Western Reserve University Center for Global Health and Disease, Cleveland, OH, United States,* [6]*University of Notre Dame, Notre Dame, IN, United States*

The fraction of *Plasmodium falciparum* infections harboring gametocytes and gametocyte densities are important indicators for human-to-mosquito transmission potential. Seasonal variation in *P. falciparum* gametocyte prevalence and density in asymptomatic individuals is not well understood. 2859 asymptomatic individuals were sampled in cross-sectional surveys in the dry season (n=1116) between January and March 2019, and wet season (n=1743) between June and August 2019 in Western Kenya, in Homabay (low transmission) and Chulaimbo (moderate transmission). *P. falciparum* infections were diagnosed by ultrasensitive varATS qPCR. Mature gametocytes were quantified using *pfs25* RT-qPCR. Parasite prevalence by qPCR was 27.0% (Chulaimbo, dry), 48.2% (Chulaimbo, wet), 9.4% (Homabay, dry), and 7.8% (Homabay, wet). Parasite density did not differ significantly between sites or seasons. *pfs25* transcripts were detected in 119/456 (26.1%) of infections, with a moderate, yet significant correlation between parasite and gametocyte density (Pearson r=0.3930, $P<0.001$). No significant difference in the proportion of gametocyte positive infections among sites was observed ($P=0.211$). In Chulaimbo, where prevalence between seasons differed substantially, the proportion of gametocyte positive infections was lower in the wet season (24.7% (50/202) vs. 38.0% (27/71), $P=0.032$), but among gametocyte-positive individuals mean *pfs25* copy numbers were significantly higher in the wet season (4.7 vs. 1.3 transcripts/uL, $P=0.018$). Age or sex were not significantly associated with gametocyte positivity or density. The proportion of gametocyte carriers was highest in school-age children in both seasons. Overall, the findings show that gametocytes persist at low density during the dry season and constitute an important reservoir for transmission. Results from Chulaimbo imply that parasites increase the investment in transmission period to adjust to vector abundance.

218

IDENTIFYING MALARIA RISK FACTORS IN CAMBODIA: UNDERSTANDING THE IMPACT OF ENVIRONMENT, MOBILITY AND BEHAVIOR ON RURAL POPULATIONS

Anais Pepey[1], Marc Souris[2], Vincent Herbreteau[3], Ivo Mueller[4], Serge Morand[5], Dysoley Lek[6], Siv Sovannaroth[6], Amélie Vantaux[1], Benoit Witkowski[1]

[1]*Pasteur Institute in Cambodia, Phnom Penh, Cambodia,* [2]*Research Institute for Development, Bondy, France,* [3]*UMR ESPACE-DEV, Montpellier, France,* [4]*Pasteur Institute of Paris, Paris, France,* [5]*French National Centre for Scientific Research, Montpellier, France,* [6]*National Centre for Parasitology Entomology and Malaria Control, Phnom Penh, Cambodia*

Environmental, ecological and geographical factors increasingly appear as crucial parameters of malaria dynamics. In South-East Asia, where forest activities correspond to the most important risk factor, these parameters remain poorly understood. Thus, we conducted a transdisciplinary study focusing on transmission patterns in a malaria-endemic area of Cambodia. First, we processed satellite imagery from 1988, 1998, 2008 and 2018 to produce land use classifications and landscape metrics of the study area. We observed an increased deforestation and fragmentation over time, as the wooded areas surface decreased from 91% in 1988 to 47% in 2018. Second, we investigated human behavior and mobility within the different environments, by using GPS data-loggers for 2 weeks as well as dried blood spots and questionnaires. Although types of visited environment varied between rainy and dry season, participants spent on average 13% of their time in forest (14h over 2 weeks), while villages were the most visited environment (average: 47.2%, 89h) and tree plantations the least visited (average: 7.9%, 15h). Over the 160 participants followed during rainy season, 30.6% were positive for malaria at baseline (Pv: 89.6%, Pf: 6.3%, mixed: 4.2%), whereas 26.3% were positive during dry season (200 participants; Pv: 82.4%, Pf: 1%, mixed: 2%). Preliminary results showed no correlation between time spent in forest and malaria status in the followed participants. However, the visits of rice fields were correlated to higher risk of malaria (P-value = 0.048, OR = 1.7) and often occurred with family members (20.3% with children, 33.8% with a female relative). Third, we performed quarterly mosquito collections from October 2019 to August 2020 to understand malaria vector bionomics in the same study area. Preliminary data (3,571 mosquitoes) shows that the abundance and diversity of *Anopheles* varied among the different types of land use, the intensity of human passage and the time of the day. Overall, using a multidisciplinary approach at a microscale shed new light on malaria dynamics and will help design tailor-made control strategies compatible with Cambodian context.

219

NATIONAL ESTIMATES OF MALARIA INFECTION IN OLDER CHILDREN AND ADOLESCENTS IN NIGERIA REVEAL HIGHEST PREVALENCE IN 5 TO 9-YEAR-OLDS

Laura C. Steinhardt[1], Ado Abubakar[2], Perpetua Uhomoibhi[3], Mark Maire[4], Stacie Greby[4], Orji Bassey[4], Nnaemeka Ndodo[5], Gretchen Cooley[1], Ryan Wiegand[1], Bala Mohammad Audu[1], McPaul Okoye[4], Matthias Alagi[4], Nnaemeka Iriemenam[4], Abiodun Ogunniyi[5], Mahesh Swaminathan[4], Chikwe Ihekweazu[5], Eric Rogier[1]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*IHVN, Abuja, Nigeria,* [3]*Ministry of Health, Abuja, Nigeria,* [4]*Centers for Disease Control and Prevention, Abuja, Nigeria,* [5]*Nigeria Centre for Disease Control, Abuja, Nigeria*

Nigeria accounted for an estimated 25% of the world's malaria cases in 2018. However, nationally representative data are only available for children <5 years old, and malaria prevalence and transmission patterns among older ages are largely unknown. Dried blood spots collected during a large national HIV household survey in 2018 were assayed for malaria antigens by a bead-based platform to detect presence of: *Plasmodium falciparum* histidine-rich protein II (HRP2), pan-*Plasmodium* lactate dehydrogenase (pLDH) and pAldolase, and *P. vivax* LDH. Finite mixture models were used to determine thresholds for antigen positivity for HRP2 and pLDH. Prevalence estimates were weighted to account for sampling probability and non-response, and standard errors account for cluster sampling. Among 30,249 specimens from children <15 years old, 47.2% (95% CI: 45.8, 48.5) were positive for HRP2 antigen, indicating current or recent infection: 40.1% (95% CI: 38.5, 41.7) of 0-4-year-olds, 52.1% (95% CI: 50.3, 53.8) of 5-9-year-olds, and 48.3% (95% CI: 46.8, 49.8) of 10-14-year-olds. Among children <15 years old, antigen prevalence was highest in the North West Zone (60.3%) and lowest in the South West Zone (30.2%), with a wide range among States (highest in Kebbi (75.8%); lowest in Lagos (7.0%)). State-level antigen prevalence among children <5 years showed good correlation ($r^2 = 0.68$) with rapid diagnostic test (RDT) positivity from the 2018 Demographic and Health Survey (36.2% overall among 6-59-month-olds), conducted around the same time. Preliminary identification of specimens for additional molecular analyses (HRP2-negative/pLDH positive) indicated low (<5%) estimated rates of non-falciparum species and little evidence for HRP2/3 deletions in the Nigerian population. Estimates of higher burdens in older children suggest that additional specific interventions targeting these groups might be warranted. Additional analyses on malaria antigen prevalence in adults, along with initial molecular results, will be presented.

220

BASELINE INCIDENCE OF POTENTIAL ADVERSE EVENTS, MALARIA, MENINGITIS AND MORTALITY IN SUB-SAHARAN AFRICAN INFANTS AND YOUNG CHILDREN: INTERIM RESULTS OF THE RTS,S/AS01$_E$ PRE-VACCINE INTRODUCTION COHORT STUDY

Kwaku Poku Asante[1], the RTS,S Epidemiology EPI-MAL-002 study group

[1]*Kintampo Health Research Centre, Kintampo, Ghana*

Despite remarkable progress in reducing the global burden of malaria, morbidity and mortality due to the disease remain very high, especially in sub-Saharan Africa. In 2019, the RTS,S/AS01$_E$ malaria vaccine was introduced in selected areas of Ghana, Kenya and Malawi through national immunization programmes in the framework of the WHO-commissioned Malaria Vaccine Implementation Programme (MVIP). The RTS,S epidemiology group is conducting several studies in the pilot implementation areas to assess vaccine safety, effectiveness and impact. The lack of baseline disease incidence rate figures in those areas may hamper this assessment. For this reason, these studies include a before-after comparison of data prior to and after vaccine introduction. The studies are observational and follow a cohort study design to estimate incidence rates of malaria, meningitis and other pre-defined rare diseases that may be reported as adverse events following vaccination, other events that lead to hospitalisation, and mortality in children less than 5 years of age. Results of interim analysis of the pre-vaccine introduction study (NCT02374450; EPI-MAL-002 study) conducted in Ghana and Kenya are presented here. Depending on the level of diagnostic certainty (clinically suspected, probable and/or etiology confirmed meningitis), the baseline meningitis incidence rate varied from 46 (95% CI: 6-167) to 92 (95% CI: 25-236) per 100,000 person-years. The incidence rate of malaria, severe malaria and cerebral malaria were 47,824 (95% CI: 45,411-50,333), 1,919 (95% CI: 1,461-2,476) and 33 (95% CI: 1-181) per 100,000 person-years, respectively. The all-cause mortality rate was 969 (95% CI: 699-1,310) per 100,000 person-years. The data presented here covers a short subject follow-up period; by the end of the post-vaccine introduction study, estimates and comparisons will be based on a total follow-up period of 2 years after the vaccination with the 4th dose of the vaccine.

221

FOREST-GOING AS A RISK FACTOR FOR CONFIRMED MALARIA CASES IN CHAMPASACK PROVINCE, LAO PDR

Sarah Gallalee[1], Emily Dantzer[1], Francois Rerolle[1], Andrew Lover[2], Bouasy Hongvanthong[3], Adam Bennett[1]

[1]*The Malaria Elimination Initiative, University of California, San Francisco, San Francisco, CA, United States,* [2]*University of Massachusetts Amherst, School of Public Health and Health Sciences, Amherst, MA, United States,* [3]*Center of Malariology, Parasitology, Entomology, Vientiane, Lao People's Democratic Republic*

Lao PDR has made significant progress in reducing malaria in recent years; confirmed cases fell from 46,202 in 2012 to 8,913 in 2018 (an 81% decrease). In the context of increasing resistance to artemisinin-based combination therapies (ACTs) in the Greater Mekong Subregion (GMS), the country aims to eliminate malaria by 2030. Current evidence in the GMS suggests working and sleeping in the forest are potential risk factors for malaria; this study sought to assess forest travel as a risk factor for confirmed malaria cases identified through the health system in a relatively higher burden province of Lao PDR. Routine passive surveillance data were extracted from health facilities in four districts in Champasack Province from August 2017 to December 2018; these data were augmented to include reporting of recent forest travel. The dataset comprised malaria test results, demographic information (age, gender, occupation) and forest-going frequency for patients who were tested for malaria. Logistic regression was conducted to assess the relationship between forest-going and positive malaria diagnosis, including a health facility level random effect. Out of 7,210 people tested for malaria during this period, 340 (4.7%) tested positive; of positive cases, 83% were male and 81% were between the ages of 16 and 50. 61% of cases were *Plasmodium vivax*and 38% were *Plasmodium falciparum*. 256 positive patients (75% of positives) and 2,723 negatives (40% of negatives) had forest travel data available. Of patients with forest travel data available, 99.6% of positives and 71% of negatives reported sleeping at least one night in the forest in the last 30 days while 39% of positives and 7% of negatives reported sleeping 7 or more nights in the forest. Compared with sleeping 1-2 nights in the forest, sleeping 3-6 nights in the forest led to 4.7 times the odds of malaria (95% CI: 2.69 to 8.12, P<.001), and 7 or more nights led to 17.5 times the odds of malaria (95% CI: 6.64 to 46.13, P<.001). Forest-going, especially longer trips, is associated with increased risk for malaria in Southern Lao PDR; targeted control efforts are needed to protect this population.

222

IN VITRO SUSCEPTIBILITY OF TAFENOQUINE AGAINST *PLASMODIUM FALCIPARUM* AMONG KENYAN SAMPLES

Redemptah Yeda, Edwin Mwakio, Agnes Cheruiyot, Hosea Akala

Walter Reed Project, Kisumu, Kenya

Tafenoquine, an 8-aminoquinoline with activity against all human life cycle stages of *Plasmodium falciparum* was recently approved by the US Food and Drug Administration for malaria prophylaxis. As the global community continues to embrace the use of this drug, data on the activity of this drug against field isolates is critical for complementing the limited efficacy data. 629 malaria positive samples collected between 2008 and 2019 under an approved malaria surveillance and transmission dynamics study in Kenya were tested for susceptibility to tafenoquine using a validated SYBR green I fluorescence assay. The activity of this drug against each isolate was discerned from dose-response curves generated from the per-well relative fluorescence units (RFU) using Graph Pad Prism. Reference clones chloroquine-sensitive (D6) and chloroquine-resistant (W2) strain of *P. falciparum* were tested in parallel. Data revealed median IC_{50}s value of 321.6 *ng/ml* [IQR 105.6-653 *ng/ml*, *n*= 629 (95% CI: 437.9-520)] for the entire period. Temporal analyses showed that in 2008 and 2018, the median IC_{50}s were 478.2ng/ml [IQR 150.8-1314ng/ml *n* =13] and IC_{50}s 424.3 ng/ml [IQR 190.4-624.6 *n*=79] with a P< 0.0001 while in 2009 - 2017 and 2019 data remained unchanged. Notably, the median IC50s between 2008 and 2019 were comparable, suggestive of sustained high *in vitro* activity against field isolates. IC_{50}s for controls strains were as follows D6 (352.4 *ng/ml*,) and W2 (289.9 *ng/ml*,). Currently, required Phase IV post marketing studies continue to monitor for any and all potential side effects of tafenoquine treatment, such as methemoglobin and risk of hemolysis in glucose-6-phosphate dehydrogenase deficient patients before it can be deployed in the African population. This study suggest that the use of tafenoquine would be an effective treatment against circulating strains of *P. falciparum* in Kenya, where malaria is an endemic disease, pending the results of Phase IV monitoring studies.

223

RAPID IDENTIFICATION OF GAPS IN ROUTINE HEALTH INFORMATION SYSTEMS TO STRENGTHEN MALARIA SURVEILLANCE

Debra Prosnitz, Yazoume Ye, Michael Paula

ICF, Rockville, MD, United States

Health information system (HIS) performance, including production of quality data and data use, contribute to improved healthcare delivery and health outcomes. High-functioning HIS performance includes that of routine health information systems (RHIS). MEASURE Evaluation and PMI Measure Malaria developed a set of RHIS architecture profiles as a tool to identify gaps in RHIS across 17 priority countries and to support strengthening of malaria surveillance. Our synthesis of these profiles compared key characteristics, data components, and data quality activities of RHIS and of integrated disease surveillance and response systems (IDSR) across countries. We found that almost all countries have moved to digital platforms—primarily the District Health Information Software, version 2 (DHIS2) platform—for managing HIS data, although one country uses a paper-based system at the national level and almost all countries use paper-based recording and reporting at the facility and community levels. Malaria data components captured in RHIS—though varying slightly across countries—do not include all data elements in the World Health Organization (WHO) guidance for disease surveillance for malaria control. Only 11 of the 17 countries we assessed capture malaria cases that were clinical, presumed, or unconfirmed, even though WHO highlights the need to report these separately for comparison over time. Countries also have different approaches to quality assurance of malaria data. Countries that do conduct regular data quality assessments do not have a standardized approach for the timing or level of assessment. These gaps present challenges for analysis and use of malaria data, particularly at subnational levels where analysis is important to guide operational decision making. Overall, our synthesis of country profiles suggests a need to better capture priority malaria data components beginning at the facility and community levels, and to improve data quality assurance at facilities, where paper-based recording and reporting is the norm.

224

HIGH FEMALE *PLASMODIUM FALCIPARUM* GAMETOCYTE DENSITY MODULATES PARASITE TRANSMISSION IN ASYMPTOMATIC MALARIA INFECTIONS

Raphael Okoth[1], Benjamin Opot[1], Gladys Chemwor[1], Irene Onyango[1], Gladys Kerich[1], Dennis Juma[1], David Aboum[1], Hoseah Akala[1], Ben Andagalu[1], Edwin Kamau[2], Jessica Cowden[3], Amanda Roth[4]

[1]*United States Army Medical Research Directorate-Africa (USAMRD-A), Kenya Medical Research Institute (KEMRI), Kisumu, Kenya,* [2]*U.S. Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [3]*Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [4]*United States Army Medical Research Directorate – Africa, Kisumu, Kenya*

Understanding parasite transmission dynamics is relevant to malaria control especially at a time when various regions are moving towards malaria pre-elimination phase. However, the influence of *P. falciparum* gametocyte sex ratio and density on transmission success has been inconclusive and its epidemiology poorly understood. This study assessed *falciparum* gametocyte sex-specific density and infectivity in asymptomatic infection. A total of 4214 blood samples collected from 29 clusters under a malaria transmission dynamics study in Kisumu, Kenya between July 2015 and June 2016 were diagnosed for malaria using a reverse transcription quantitative real time polymerase chain reaction (RT-qPCR). The malaria positive samples were also tested for gametocyte carriage and gametocyte stage composition based on presence of Pfs16 and Pfs25 and were fed to sterile mosquito colonies by membrane feeding assay (MFA) and/or direct landing feeding assay (DFA) to determine infectivity of gametocyte positive infections. A modifies sex-specific gametocyte quantification assay was carried out in a subset of the gametocyte positive samples and correlated to the oocyst density from the feeding assays. 56% (2375/4214) samples were malaria positive with 25% (1065/2375) of these cases having early and/or late stage gametocytes. Interestingly, 82.4% of the gametocytemic cases were submicroscopic. No significant difference in the feeding outcomes between MFA and DFA (*P=0.168*) was observed. Using Spearman's correlation, female gametocyte density had a marginal correlation with the oocyst density (*R = 0.49*) compared to male gametocytes (*R = 0.03*). The findings of this study suggest that in malaria asymptomatic populations, female gametocytes density plays a major role in propagating infection from the human host to the vector thus sustaining malaria transmission.

225

OPTIMIZING ACCESS TO RECTAL ARTESUNATE: UNDERSTANDING DRIVERS OF TREATMENT SEEKING FOR SEVERE FEBRILE ILLNESS IN CHILDREN UNDER 5 IN NIGERIA AND UGANDA

Nina Brunner[1], Aita Signorell[1], Phyllis Awor[2], Elizabeth Omoluabi[3], Theodoor Visser[4], Harriet Napier[4], Valentina Buj[5], Christian Burri[1], Christian Lengeler[1], Manuel Hetzel[1]

[1]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [2]*Makerere University School of Public Health, Kampala, Uganda,* [3]*Akena Associates, Abuja, Nigeria,* [4]*Clinton Health Access Initiative, Boston, MA, United States,* [5]*UNICEF, New York, NY, United States*

Rectal artesunate (RAS) is a potentially life-saving pre-referral drug for young children with severe malaria in settings with poor access to emergency care. Current recommendations are for community-based providers to administer RAS based on dangers signs following the integrated community case management (iCCM) algorithm, and to refer the child to the nearest higher-level health facility for complete treatment.

In reality, some children with severe febrile illness are brought directly to referral facilities without first consulting a community-based provider. While some of these children would not have been in need of RAS because of their proximity to the referral facility, children living in remote communities should first be seen by a community-based provider to ensure prompt access to this life-saving treatment before transfer to a higher-level facility. To ensure effective targeting and rational use of RAS, it is necessary to understand the drivers of these different treatment seeking patterns. In the frame of the Community Access to Rectal Artesunate for Malaria (CARAMAL) project, 2,598 children <5 years presenting to community-based providers with severe febrile illness were enrolled in an area of perennial malaria transmission in Uganda, and 473 children in an area of seasonal malaria transmission in Nigeria (April 2018-December 2019). In the same period, 1,643 children with severe malaria were enrolled in referral facilities in Uganda, and 347 in Nigeria. All children were followed up 28 days after enrolment. We investigated factors associated with first consulting a community-based provider versus directly seeking treatment from a referral facility by comparing symptoms, illness severity, distance to referral facilities, and socio-demographic characteristics of the enrolled children. The results of the comparative analysis of real-world evidence will be discussed in light of current treatment guidelines and health system regulations in two diverse settings in sub-Saharan Africa. Additionally, health system implications for the effective roll-out of RAS will be presented.

226

INVESTIGATION OF SUSPECTED EPIDEMICS OF MALARIA IN THE DEMOCRATIC REPUBLIC OF THE CONGO FROM 2004 TO 2014

Divine Dilubenzi[1], Didier Bompague[1], Gisele Mvumbi[2], Jean-Jacques Muyembe[1]

[1]*URF/University of Kinshasa/INRB, Kinshasa, Democratic Republic of the Congo,* [2]*FELTP/University of Kinshasa/INRB, Kinshasa, Democratic Republic of the Congo*

Malaria remains the first cause of morbidity and mortality in the DRC. Since 2010, outbreaks of suspected malaria have been reported outside the health districts in mountain facies. Analyzes with Excel, QGIS® (2.2.0), SaTScan v9.1.1 and prologiciel R have been developed to study suspected malaria reported by the IDSR system in the country between 2004 and 2014. From 2004 to 2014, 80,930,741 cases and 180,202 deaths (0.22% of cases) of suspected malaria were reported. During outbreaks (2010-2013), 301,769 cases, 936 deaths (0.31% of cases) have been reported in 26 countries' Health Districts.The majority of Health districts who presented the outbreaks are in stable malaria transmission areas. The sentinel surveillance system is not fully implemented. In conclusion, this work showed that in stable malaria Health Districts are at risk of occurrence of outbreaks of epidemics suspected malaria.However, it will be useful to conduct additional studies to improve understanding of the factors underlying these epidemics.

227

INVESTIGATION OF DEATHS IN MALARIA-ENDEMIC COUNTRIES: FINDINGS OF AN AUDIT OF MALARIA-RELATED DEATHS IN BENIN

Migan Theotime, Abou Bakary Pio, Rodrigue Achille Couao-Zotti, Christian Godjo, Sakariaou Kpanou, Cyriaque Dossou Affoukou, Aurore Ogouyemi-Hounto

Programme Notional de Lutte contre le Paludisme, Cotonou, Benin

National guidelines on reporting malaria death were established in 2010 in Benin. By the end of 2018, an estimated number of 2151 of malaria-related deaths were recorded. To ensure that these deaths are attributable to malaria, the National Malaria Control Program (NMCP) investigated the deaths recorded nationwide. The investigation aimed, on the one hand at ascertaining the true proportion of malaria-related deaths among death reported in hospitals and health facilities in comparison with the data recorded on medical records, and on the other hand at identifying the characteristics of validated death cases. After reviewing medical records, 1066 cases were validated as malaria-related deaths, representing 54% of the 1953 total number of reported deaths recorded in hospitals and health facilities in Benin. Of the 1066 validated malaria-related cases, 52% were males, 83.9% of cases were confirmed by The Thick Drop with a positivity rate of 79.8%. The average age of validated cases was 44, 2 months or 3.7 years and the average length of hospital stay is 1.05 days. Of the 887 cases (45.41%) invalidated deaths, 10.5% occurred at the entrance to the hospital, 9.5% were tested negative without treatment, 13.6% have not been tested for malaria, 9.8% of the specified diagnoses were non-malaria, 70.9% of the malaria cases had no diagnosis, and 4.2% are not accompanied by any signs of malaria severity. The cost of hospitalization and medication for 71% of the validated deaths cases was covered by the patient. 68% received malaria treatment. Our investigation showed an overestimation of malaria-related deaths. Poor knowledge of national reporting guidelines may explain the inaccurate number of reported malaria-specific deaths in Benin, however poor adherence to severe malaria treatment guidelines likely contributes to high malaria mortality as well. Care providers should be better trained on the optimal implementation of the national guidelines for the management of severe malaria. Data managers must be trained to properly identify malaria-related deaths to help improve the malaria-related deaths data quality.

228

SEX-BASED DIFFERENCES IN CLEARANCE OF CHRONIC *PLASMODIUM FALCIPARUM* INFECTION

Jessica Briggs[1], Noam Teyssier[1], Joaniter Nankabirwa[2], John Rek[2], Prasanna Jagannathan[3], Emmanuel Arinatiwe[1], Teun Bousema[4], Chris Drakeley[5], Margaret Murray[3], Emily Crawford[6], Nicholas Hathaway[7], Sarah Staedke[5], David Smith[8], Phillip J. Rosenthal[1], Moses Kamya[9], Grant Dorsey[1], Isabel Rodriguez-Barraquer[1], Bryan Greenhouse[1]

[1]*UCSF, San Francisco, CA, United States,* [2]*IDRC, Kampala, Uganda,* [3]*Stanford, Palo Alto, CA, United States,* [4]*Radboud University, Nijmegen, Netherlands,* [5]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [6]*Chan-Zuckerberg Biohub, San Francisco, CA, United States,* [7]*University of Massachusetts Amherst, Amherst, MA, United States,* [8]*Institute for Health Metrics and Evaluation, Seattle, WA, United States,* [9]*Makerere University College of Health Sciences, Kampala, Uganda*

Multiple studies have reported a higher prevalence of malaria infection in males compared to females. However, it remains unknown whether this is due to differences in behavioral factors or biological sex playing a direct role in the host response to the malaria parasite. To test the hypothesis that sex-based differences in host-parasite interactions affect the epidemiology of malaria, we intensively followed a cohort of individuals living in a malaria endemic area of eastern Uganda. By performing frequent sampling, ultrasensitive quantitative PCR (qPCR), and amplicon deep sequencing, we followed *Plasmodium falciparum* infections over time to estimate both force of infection (FOI) and rate of clearance by sex. Prevalence of malaria infection by qPCR was 14.4% in males versus 9.2% in females (difference 5.2%, 95% confidence interval [CI] 3.8% to 6.5%). There was no evidence of differences in behavioral risk factors, incidence of malaria, or FOI by sex. In contrast, females cleared asymptomatic infections at a faster rate than males (hazard ratio [HR] = 1.73, 95% CI 1.13 to 2.65 by clone and HR = 2.07, 95% CI 1.24 to 3.47 by infection event) in multivariate models adjusted for age, timing of infection onset, and parasite density. In conclusion, differences in *P. falciparum* prevalence between males and females observed in endemic settings were driven by faster clearance rates in females and not by increased infection rates in males. These findings implicate biological sex-based differences as an important factor in the host response to this globally important pathogen.

229

DISENTANGLING THE EFFECTS OF CLIMATE, DEFORESTATION, AND CONTROL EFFORTS ON MALARIA EPIDEMIOLOGY IN THE PERUVIAN AMAZON RAINFOREST

Gabriel Carrasco-Escobar[1], Marcia C. Castro[2], Mercedes Pascual[3], Isabel K. Fletcher[4], Cesar Cárcamo[1], Miguel Campos[1], Hugo Rodriguez[5], Cristiam Carey[5], Jan Conn[6], Alejandro Llanos-Cuentas[1], Rachel Lowe[4]

[1]*Universidad Peruana Cayetano Heredia, Lima, Peru,* [2]*Department of Global Health and Population, Harvard T.H. Chan School of Public Health, Boston, MA, United States,* [3]*Department of Ecology and Evolution, University of Chicago, Chicago, IL, United States,* [4]*Centre for Mathematical Modelling of Infectious Diseases, London School of Hygiene & Tropical Medicine, London, United Kingdom,* [5]*Dirección Regional de Salud Loreto, Loreto, Peru,* [6]*Wadsworth Center, New York State Department of Health, Albany, New York, NY, United States*

The Peruvian Amazon is a region undergoing rapid changes in malaria epidemiology as a result of climate and complex land alterations, including deforestation. This study develops a spatio-temporal Bayesian modeling approach to disentangle the multiple drivers of malaria risk and quantify the extent to which variations in climate, land use, and control efforts are associated with *Plasmodium vivax* and *P. falciparum* malaria incidence rates in the Peruvian Amazon. We gathered monthly data from the malaria surveillance system of the Ministry of Health, malaria control activities, and remotely-sensed data on land use and climatic factors for 18 years (Jan 2000 - Dec 2017) for all the districts (49) in the Loreto Region. High spatial heterogeneity was observed with pockets of high malaria transmission in the north and south of the study area and a sharp reduction in the *P. vivax* and *P. falciparum* cases during the intensified malaria control period between 2006 and 2010.. Our findings suggested that a different set of control activities were suitable for each malaria species. Overall, models that included nonlinear terms of maximum temperature, precipitation, and deforestation outperformed the estimation of *P. vivax* and *P. falciparum* malaria. Deforestation showed an increasing effect on *P. vivax* and *P. falciparum*, with a peak at 150 km^2. The results of this study highlight the key role of deforestation to condition the malaria infection and we provided the first evidence to support the frontier malaria framework in the Peruvian Amazon. Finally, this study provides insights into the added value of accounting for non-linear effects of climate and deforestation variables to improve the estimation of malaria incidence in a spatially-heterogenous transmission.

230

DECREASED MORTALITY OF FALCIPARUM MALARIA IN ANEMIC PRISONERS OF WAR?

G Dennis Shanks

Australian Defence Force Malaria and Infectious Disease Institute, Enoggera, Australia

Falciparum malaria mortality rates vary depending on adequacy and speed of treatment as well as host factors. Extreme examples of malaria mortality were sought in the military medical records of the Second World War in Asia. Hospital case fatality rates <1% were typical of falciparum malaria with two exceptions. When Imperial Japanese Navy sailors captured on Nauru (n=799) were imprisoned on the Fauro Islands, 26% died falciparum malaria despite similarly treated Imperial Army soldiers showing no change in mortality. One-fifth of Australian Army soldiers (n=252) retreating from New Britain died largely due to malaria in April 1942. Malnourished prisoners of war both Australian Army soldiers in Thailand and Japanese Army soldiers in Papua New Guinea had high malaria rates but very low mortality rates. Anemia has been suggested as a possible protective factor against severe falciparum malaria. Malaria immunity does not adequately explain this dichotomy suggesting that severe nutritional deprivation may be protective against malaria mortality possibly due to iron-deficiency anemia.

231

ASSESSING MALARIA DATA REPORTING ACCURACY IN THE NORTH AND FAR NORTH REGIONS IN CAMEROON: ANALYSIS OF DATA FROM A RAPID DATA QUALITY ASSESSMENT

Olivier M. Kakesa

MEASURE Evaluation, Yaounde, Cameroon

Since 2018, Cameroon's National Malaria Control Program (NMCP) has routinely collected and reported monthly malaria case data through the District Health Information Software, version 2 (DHIS2) platform. Twenty-six NMCP staff were trained in November 2019 on data quality assurance and data reporting tools provided to health facilities. To assess the quality of data reported, a data quality assessment was conducted using a Rapid Data Quality Assessment (RDQA) tool in selected health facilities in the North and Far North regions of Cameroon in March 2020. Two hundred and eleven health facilities were visited that month (111 in North and 100 in Far North regions). This analysis compared data accuracy between the two regions for selected malaria indicators, including the number of suspected cases, the number of cases tested, and the number of confirmed cases. Data reported to DHIS2 between November 2019 and January 2020 were compared with data from outpatient registers using a verification ratio (VR). A VR of 1 indicated accurate reporting from outpatient registers to DHIS2, while a VR less than or more than 1 indicated inaccurate reporting. We set a benchmark of at least 80% of data matching between the Outpatient Department register and DHIS2. For the number of suspected malaria cases, 84.7% of health facilities (HF) in the North region reported more than 80% accuracy compared to 65% of HF in the Far North region (p-value<0.0001). Regarding the number of malaria cases tested, reporting accuracy was similar in both regions (62% vs 72%, p-value=0.1297). Similarly, the reporting accuracy for the number of confirmed malaria cases was comparable in both regions (North=52%, Far North=54%, p-value=0.8259). On average, 73% of health facilities showed underreporting from outpatient registers to DHIS 2. This assessment indicates that overall, HFs in the North and Far North regions are underreporting data in DHIS2, and have reporting accuracies that vary depending on the indicator and the region. These results serve as a baseline for measuring data quality improvement overtime as capacity strengthening is provided to NMCP staff based on the gaps identified.

232

FOREST MALARIA IN CAMBODIA: THE OCCUPATIONAL AND SPATIAL CLUSTERING OF *PLASMODIUM VIVAX* AND *P. FALCIPARUM* INFECTION RISK IN A CROSS-SECTIONAL SURVEY IN MONDULKIRI PROVINCE, CAMBODIA

Mirco Sandfort[1], Amélie Vantaux[2], Saorin Kim[2], Thomas Obadia[1], Anaïs Pepey[2], Soazic Gardais[1], Nimol Khim[2], Dysoley Lek[3], Michael White[1], Leanne J. Robinson[4], Benoit Witkowski[2], Ivo Mueller[1]

[1]*Malaria: Parasites and Hosts unit, Institut Pasteur, Paris, France,* [2]*Malaria Molecular Epidemiology unit, Institut Pasteur of Cambodia, Phnom Penh, Cambodia,* [3]*National Center for Parasitology, Entomology, and Malaria Control, Phnom Penh, Cambodia,* [4]*Population Health & Immunity, Walter and Eliza Hall Institute, Melbourne, Australia*

After a marked reduction in malaria burden in Cambodia over the last decades, the country faces increasing case numbers after a trough in 2016. In light of the national goal of malaria elimination by 2025, remaining pockets of high risk need to be well defined and strategies tailored to identify and target the persisting burden cost-effectively. In a cross-sectional survey in the Mondulkiri province in Cambodia from December 2017 until April 2018, 4200 randomly selected participants were tested for *Plasmodium spp.* infections by PCR. *P. vivax* predominated over *P. falciparum* with a prevalence of 6.8% and 3.3% (p<0.001), respectively. 2.2% and 7.2% of the *P. vivax* and *P. falciparum* PCR-positive infections were febrile and light microscopy or RDT-positive, i.e. they could be detected by the health system. Infections are concentrated in men, with highest prevalence in occupationally active age groups (peaking at 21.4%

and 11.1% in 21-25 years old for both species, respectively, $p<0.001$). Recent travels to forest sites (aOR 2.13, $p<0.01$) and forest work (aOR 2.36, $p<0.001$) were particularly strong risk factors, with comparable profiles for both species. Large village-level differences in prevalence of *Plasmodium spp.* infection were observed, spanning values as low as 0.6% outside the forest to 40.4% in forested areas. Residing in villages inside the forest was a strong spatial risk factor (aORs 8.82, $p<0.001$). Analysis of the spatial distribution of malaria cases using the spatial signature statistical method revealed widespread and significant clustering of malaria cases within a 50 m radius. The survey sheds light on the predominance of *P. vivax* and the persistence of social and spatial pockets of high prevalence in Cambodia. The spatial signature allows to generate hypotheses for radius-based reactive control interventions in a locally adapted manner which are potentially cost-effective tools for the last mile to malaria elimination.

233

EVALUATING THE ASSOCIATIONS BETWEEN LOW-DENSITY *PLASMODIUM FALCIPARUM* INFECTIONS, HIGHER-DENSITY INFECTIONS, AND POSSIBLE IMPORTED INFECTIONS ON BIOKO ISLAND, EQUATORIAL GUINEA

Dianna E. Hergott[1], Kurtis R. Cruz[1], Haley Masters[1], Irene Cruz Talavera[1], Annette M. Seilie[1], Jokichi Matsubara[1], Aveika Akum[2], Guillermo A. García[3], Brandon L. Guthrie[1], Jennifer E. Balkus[1], Sean C. Murphy[1]

[1]*University of Washington, Seattle, WA, United States,* [2]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [3]*Medical Care Development International, Silver Spring, MD, United States*

Despite control efforts, the prevalence of *Plasmodium falciparum* (*Pf*) on Bioko Island, Equatorial Guinea was 18% in 2019. Control may be hindered by (1) low-density infections (LDIs) that are transmissible but not detected by rapid diagnostic tests (RDTs); and (2) imported infections acquired off the island and re-introduced and propagated locally. The prevalence of LDIs and their impact and that of imported infections to transmission in Bioko is unknown. We analyzed dried blood spots (DBS) from the 2015 Bioko Island Malaria Indicator Survey by quantitative reverse transcription PCR (qRT-PCR) to detect *Pf* LDIs and used results to test for associations between LDIs, residence in a household with an RDT+ individual, and recent travel by RDT+ persons. We selected 132 households with an RDT+ individual and matched them 1:4 to households without RDT+ persons. Multilevel logistic regression models that incorporated inverse probability weights to account for possible selection bias in household sampling were used. Models were adjusted for known confounders and accounted for clustering at the household and locality level.Of 1780 samples from RDT- individuals in 582 households, 173 (9.7%) harbored LDIs by qRT-PCR (mean parasite density 122 p/μL). The adjusted odds of finding LDIs in households with an RDT+ individual was 2.1-fold greater than in homes without RDT+ individuals (95% CI: 1.1-4.0). Travel by RDT+ individuals in the past eight weeks was not associated with higher prevalence of LDIs in the household (aOR=1.32, 95% CI: 0.30-5.86). In households without reports of recent travel, the odds of finding LDIs in homes with an RDT+ individual was 2.4-fold greater than in those without an RDT+ individual (95% CI: 1.13-4.95). These results suggest that there are a large proportion of LDIs on Bioko Island, and that LDIs are more common in households with RDT+ individuals who have not recently traveled. Implementation of household-based test-and-treat approaches reliant on RDTs may target transmissible LDIs as well, especially in areas with low risk of importation.

234

MECHANISMS DRIVING THE SPREAD OF HISTIDINE-RICH PROTEIN 2 OR 3 DELETIONS - A MODELING APPROACH

Pierre Marie Ngougoue Ngougoue, Kristan Alexander Schneider

Hochschule Mittweida, University of Applied Sciences, Mittweida, Germany

One of the cornerstones of controlling P.falciparum malaria is the use of rapid diagnostic tests (RDTs), recommended to confirm infections prior to treatment with ACTs as a measure of containment of artemisinin resistance. *P. falciparum* histidine-rich proteins 2 and 3 (pfhrp2 and pfhrp3) emerged as the most appropriate and popular antigens targeted by RDTs. However, increasing prevalence of parasites with pfhrp2 and/or pfhrp3 deletions is severely challenging proper RDT-based diagnostics by yielding false-negative results, potentially leading to improper treatment and an undetected reservoir for malaria transmission. Understanding the evolutionary process underlying the origin and the spread of HRP gene deletions is urgently needed to sustain reliable and cost-efficient diagnostics. The impact of the potential evolutionary mechanisms can be studied by mathematical models tailored to the specifics of malaria transmission, characterized by the presence of genetically distinct parasites haplotypes within infections due to multiple infective contacts (multiplicity of infection, MOI). We introduce a deterministic population-genetic model to study the evolutionary dynamics of HRP2 or HRP3 deletions. The model is tailored to the characteristics of the transmission cycle of *P. falciparum*. Particularly, the interplay between transmission intensity (MOI) and the spread of deletions is explained in detail. The model shows that selection on HRP deletions originating only from delayed treatment due to false-negative RDT results is extremely weak on emerging haplotypes with deletions, particularly in high transmission areas. The mechanism of selection is more effective in areas of low transmission, or if parasites with HRP deletions already reached appreciable frequencies, either by drift or other mechanisms of selection. The model can be readily adapted to interpret empirical patterns of selection on HRP deletions due to delayed treatment of false-negative-tested patients or reconstruct the underlying evolutionary processes by reverse engineering. It is further employable for study design purposes.

235

APPRECIATING THE COMPLEXITY OF LOCALIZED MALARIA RISK IN GHANA: SPATIAL DATA CHALLENGES AND SOLUTIONS

Sandra O. Bempah[1], Andrew Curtis[2], Gordon Awandare[3], Jayakrishnan Ajayakumar[2]

[1]*Kent State University, Kent, OH, United States,* [2]*Case Western Reserve University, Cleveland, OH, United States,* [3]*West African Center for Cell Biology of Infectious Pathogens, Accra, Ghana*

Various factors have been associated with the ongoing high prevalence of malaria in Ghana. Among these are poor sanitation, low socioeconomic status (SES), building construction and other proximate micro environmental risks, and individual behaviors. What makes the curbing of malaria more challenging, is that for many of the most impacted areas there is little data for modeling or predictions, which are needed, as risk is not homogenous at the sub-neighborhood scale. In this study we use available local surveillance data combined with novel on-the-ground fine scale environmental data collection, to gain an initial understanding of malaria risk for the Teshie township of Accra, Ghana. Mapped environmental risk factors include open drains, stagnant water and trash. Overlaid onto these were clinical data of reported malaria cases collected between 2012 and 2016 at LEKMA hospital. We then enrich these maps with local context using a new method for malaria research, spatial video geonarratives (SVGs). These SVGs provide insights into the underlying spatial-social patterns of risks, to reveal where traditional data collection is lacking, and how and where to develop local intervention strategies. We also revealed the environmental, spatial and behavioral intersections that

can be used to understand, and map, that heterogeneity of risks. In so doing, it provides the first step in improving local data gathering to provide the operational support needed to start to make reductions in malaria risk across the communities.

236

REACHABLE OUTCOMES OF COMBINING MULTIPLE INTERVENTIONS FOR CONTROL OF MALARIA AND DRUG RESISTANCE: MODELS, ANALYSIS, AND A FRAMEWORK FOR ADAPTIVE PROGRAMS

Yael Evelyn Marshall

Princeton University, Princeton, NJ, United States

How might we take advantage of the currently available anti-malarial interventions to control malaria and drug resistance? How might we intentionally design an adaptive intervention program which efficiently improves over time? We propose a framework for multi-intervention adaptive programs for malaria and drug resistance control which incorporates (1) statistical engineering methods, (2) mechanistic epidemiological models, and (3) intervention-focused operational research; it also contains similarities to ecology methods and to past successful anti-malarial programs. To illustrate the model component, we develop simple compartmental models for low endemicity, analytical approximations, a novel framework of modular compartmental models for low and high endemicity, and a stochastic microsimulation (latter with only preliminary results). Details simulated include transmission, superinfection, immunity dynamics, drug resistance emergence and/or spread, and multiple interventions (drug treatment, indoor residual spraying of insecticides, larval habitat reduction, and either insecticidal-treated nets or a simplified homogeneous representation of barriers such as housing features). Our model analysis systematically explores the large space of possible multi-intervention programs (range of coverage combinations) and their possible effects on malaria burden and drug resistance over time. Our models predict that interventions can combine constructively to reduce burden. Interestingly, except for the simple analytical approximations, our models also predict that resistance depends not only on the fraction of clinical infections treated but also on vector-related interventions in a manner possibly depending on transmission settings, overall coverage, and whether focus is resistance spread or also emergence. Our modular compartmental models predict how, along with some possible caveats to avoid, vector-related interventions, in a program with drug treatment, might be used to generate possible advantages with respect to both reducing malaria burden as a priority and slowing the spread of drug resistance.

237

MODELLING MALARIA IN NEAR ELIMINATION SETTINGS USING HAWKES PROCESSES

H. Juliette T. Unwin[1], Seth Flaxman[1], Isobel Routledge[2], Shengjie Lai[3], Swapnil Mishra[1], Samir Bhatt[1]

[1]*Imperial College, London, United Kingdom,* [2]*University of California, San Francisco, CA, United States,* [3]*University of Southampton, Southampton, United Kingdom*

Since the year 2000, one-fifth of the then malaria-endemic countries have eliminated local malaria transmission. In 2016, the World Health Organisation identified 21 countries with the potential to eliminate malaria by 2020; seven of these countries have been successful. Research now suggests that malaria eradication within a generation is achievable but there needs to be a global commitment to achieve eradication. Modelling malaria in low transmission settings is challenging as the number of infected bites a person receives is highly heterogeneous. This greatly limits the use of gold standard measures such as parasite prevalence as prohibitively large sample sizes are needed. In this work, we propose a class of semi-mechanistic models known as Hawkes Processes, and use them to capture malaria disease dynamics in near elimination settings. Hawkes Processes are mathematically well grounded, but they have rarely been used in epidemic scenarios. Our model combines malaria specific information within a rigorous statistical framework to fit to case incidence data, which is generally easily available through routine surveillance. We consider contributions to the force of infection from both local and imported sources separately, and can therefore easily identify the underlying mechanism that is driving transmission. We show that not only is it possible to accurately recreate the case counts over time, but it is possible to accurately predict the proportion of cases that are imported for China and Swaziland. We also show that this model is robust for forecasting. We believe our model provides a useful alternative to individual based models when empirical performance is needed.

238

ESTIMATING THE TIMING INTERVAL FOR MALARIAL INDOOR RESIDUAL SPRAYING: A MODELLING APPROACH

Levicatus Mugenyi[1], Joaniter I. Nankabirwa[1], Emmanuel Arinaitwe[2], John Rek[2], Niel Hens[3], Moses R. Kamya[1], Grant Dorsey[4]

[1]*Makerere University College of Health Sciences, Kampala, Uganda,* [2]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [3]*I-Biostat, Data Science Institute, Hasselt University, Hasselt, Belgium,* [4]*Department of Medicine, University of California, San Francisco, CA, United States*

Indoor residual spraying (IRS) reduces vector densities and malaria transmission, however, the most effective spraying intervals for IRS have not been well established. We estimated the optimal timing interval for IRS using a modelling approach. Generalized additive models were used to estimate the optimal timing interval for IRS based on the predicted malaria incidence. The model was applied to clinical data from a cohort of children aged 0.5-10 years from selected households in Tororo District, a historically high malaria transmission setting in Uganda. Six rounds of IRS were implemented in Tororo during the study period (3 rounds with bendiocarb: December 2014 to December 2015, and 3 rounds with actellic: June 2016 to July 2018). Monthly incidence of malaria from October 2014 to February 2019 decreased from 3.25 to 0.0 per person-years in the children under 5 years, and 1.57 to 0.0 for 5-10 year-olds. The optimal time interval for IRS differed between bendiocarb and actellic and by IRS round. It was estimated to be 17 and 40 weeks after the first round of bendiocarb and actellic, respectively. After the third round of actellic, 36 weeks was estimated to be optimal. However, we could not estimate from the data the optimal time after the second and third rounds of bendiocarb and after the second round of actellic. The amount of rainfall did not influence trends in malaria incidence after IRS as well as the IRS timing intervals. In conclusion, in our setting, to sustain the effect of IRS, the second rounds of IRS with bendiocarb need to be applied roughly 17 weeks and actellic 40 weeks after the first round, and the timing differs for subsequent rounds. Though, these shorter intervals than is the practice may improve the effectiveness of IRS, one should consider the cost and insecticide resistance. We also recommend that the timing and incidence should be monitored in the future to improve these estimates.

239

EVALUATING THE IMPACT OF SINGLE LOW-DOSE PRIMAQUINE ON REDUCING MALARIA TRANSMISSION IN A SOUTH AFRICAN CONTEXT

Jared Michael Norman, Sheetal Silal

University of Cape Town, Cape Town, South Africa

The estimated global annual number of deaths due to malaria in 2018 was 407,000. The WHO recommends the use of Single Low-Dose Primaquine (SLDP) to decrease transmission of the *Plasmodium falciparum* parasite. In South Africa, trials are underway to evaluate the impact of SLDP in combination with Artemether / Lumefantrine in the Nkomazi and Bushbuckridge Local Municipalities in the Mpumalanga Province. A nonlinear ordinary differential equation compartmental model was developed to simulate the transmission dynamics in these populations and estimate the intervention's impact on malaria incidence. Particular attention was paid to capturing the *in vitro* gametocyte stage of the

parasite life cycle by accounting for sequestration and maturity of gametocytes. In order to isolate the contribution of SLDP in reducing transmission, it was also necessary to simulate the impact of indoor residual spraying activities, active case detection, importation of malaria and the seasonality of transmission. The model was calibrated to data from the Mpumalanga Malaria Control Programme (MMCP) in order to estimate the cases and deaths averted due to the introduction of SLDP. The impact of SLDP at various coverage levels within the malaria season was also explored. These results of the study will be communicated to programme managers in the MMCP and may also be of relevance to malaria policy makers on the African continent.

240

SUPPORTING DECISION-MAKING FOR MALARIA ELIMINATION ON THE AFRICAN CONTINENT

Sheetal Prakash Silal[1], Jared Norman[1], Rachel Hounsell[1], Chacha Issarow[1], Wanja Chabaari[1], Timothy Awine[1], Akindeh Nji[2]

[1]*University of Cape Town, Cape Town, South Africa,* [2]*University of Yaounde, Yaounde, Cameroon*

Malaria elimination remains high on the political and funding agenda throughout the African contintent. The heterogeneous transmission landscape, variations in population and vector behaviour and the range of interventions available result in there being no "one size fits all" strategy for malaria elimination on the continent. Mathematical modelling may be one of the only available approaches for combining the many interacting factors that must be considered when designing an elimination strategy. To this end, a computer application has been developed to allow policy-makers to test out the impact of interventions and design policies by running simple mathematical models and navigating the output of millions of simulations of more complex models with the aid of interactive graphs. This application has been used to support decision-making in South Africa, Ghana and Cameroon. Questions on the historical relative impact of interventions on malaria transmission, projections at a sub-national level, and designing and costing an elimination strategy have been explored using this tool. Use of the tool has accelerated the impact that mathematical models have in turning national surveillance data into strategic information to support the policy-makers in programme and funding decisions.

241

MODELING AND COMPARING RELEASE-SCENARIOS FOR GENETICALLY ENGINEERED MOSQUITOES IN SÃO TOMÉ & PRÍNCIPE

Hector Manuel Sanchez

University of California Berkeley, Berkeley, CA, United States

CRISPR/Cas9-based technologies have revitalized interest in gene-editing technologies as means to control mosquito-borne diseases. Amongst candidate disease-control mechanisms, gene-replacement strategies are considered some of the most promising due to their resilience to generation of resistant alleles (caused by errors in homology-directed repair). These approaches focus on releasing and introgressing genes that prevent mosquitoes from transmitting pathogens to humans, thus disrupting the transmission chain.Genetically isolated populations provide perfect testing grounds to assess the viability of genetic-modification constructs, as they limit the probability of the drive escaping the area of study whilst also restricting the impact of heterogeneity in variables such as migration and spatial structure. The islands of São Tomé and Príncipe, in the equatorial region west of the African mainland are a couple of such settings that are being considered as potential testing grounds for gene drive studies.In this work, we explore release strategies to introgress a transgenic construct in a mosquito population using our published gene-drive model: MGDrivE. In doing so, we find that even though there is a benefit in increasing the ratio of the releases of transgenic mosquitoes (with respect to the natural population), a limit exists above which performing more releases does not significantly increase the speed at which the transgene takes over the population. Additionally, we find that changes in the genetic standing variation do not affect the time to introgression; and that, even though sex of the released mosquitoes (mixed, gravid, or male-only) does impact the extent of the effects, their viability has to be assessed with respect to the necessary mosquito sex-sorting labor.

242

MODELING AND SIMULATION OF LUMEFANTRINE PHARMACOKINETICS IN HIV-INFECTED AND HIV-UNINFECTED CHILDREN WITH MALARIA AND THE ROLE OF LUMEFANTRINE EXPOSURE AS A POTENTIAL DRIVER OF DRUG RESISTANCE

Katherine Kay[1], Justin Goodwin[2], Norah Mwebaza[3], Adam T. Lafontaine[1], Hanna Ehrlich[2], Joyce Ou[2], Tracy Freeman[2], Martina Wade[2], Liusheng Huang[4], Kaicheng Wang[2], Fangyong Li[2], Francesca T. Aweeka[4], Matthew M. Riggs[1], Richard Kajubi[3], Sunil Parikh[2]

[1]*Metrum Research Group, Tariffville, CT, United States,* [2]*Yale School of Public Health, New Haven, CT, United States,* [3]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [4]*University of California, San Francisco, CA, United States*

Treating malaria in children with and without HIV infection requires consideration of complex biological and pharmacological factors that impact artemisinin-based combination therapies (ACTs). Developmental changes in pharmacokinetics (PK) are often ignored, and concomitant anti-retroviral therapy (ART) results in drug-drug interactions (DDI) that may have significant effects. Drug exposure may also impact drug resistance selection. We have shown efavirenz (EFV) reduced exposure to both artemether (AR) and lumefantrine (LF) by 2.1- to 3.4-fold; lopinavir/ritonavir (LPV/r) increased LF exposure by 2.1-fold; and nevirapine (NVP) reduced AR exposure. We developed a population PK/PD model to explore the relationship between LF exposure and resistance selection, which has not been extensively evaluated. The PK model was developed in children receiving AL alone or with an ART (EFV, LPV/r, or NVP) and parameters were estimated using nonlinear mixed effects modelling (NONMEM®). The PK model consistently predicted the observed LF profiles in pediatric patients, with and without ART, as estimated by comedication effects on LF bioavailability and systemic clearance. LF exposure was estimated with the PK model and used to develop a PK-PD model that associated mutation status with recurrent infection. Recurrent infections were genotyped to classify recrudescent or new infection. Drug resistance was assessed through genotyping at pfmdr1 N86Y, pfmdr1 Y184F and pfcrt K76T, demonstrating that mutations associated with reduced susceptibility to LF (pfmdr1 N86 and pfcrt K76T) were more prevalent in recurrent infections ($p=0.004$ and <0.001, respectively). The DDI, affected by concomitant administration of LF with and without ART, provided an opportunity to evaluate a much broader LF exposure range than typically observed following standard LF dosing. This allowed for exploration of LF exposure in a high transmission area and the likelihood of mutation selection upon reinfection. Final results will be presented, allowing for further optimizing of AL dosing regimens and characterization of the impact of exposure on resistance selection.

243

A FORWARD-TIME SIMULATION OF *PLASMODIUM FALCIPARUM* TRANSMISSION AND EVOLUTION

Jason A. Hendry, Dominic Kwiatkowski, Gil McVean

University of Oxford, Oxford, United Kingdom

There is a growing abundance of malaria genetic data being collected from the field, yet relating this genetic data to regional epidemiology remains a challenge. One issue is a paucity of models relating parasite genetic diversity to epidemiological parameters. Classical models in population genetics characterize changes in genetic diversity and demography, but fail to account for the unique features of the malaria

life cycle. Epidemiological models, like the Ross-Macdonald, capture malaria transmission dynamics but fail to include genetics. Here, we have developed a new model encompassing both parasite evolution and regional epidemiology. We achieve this by combining the Ross-Macdonald model with an intra-host continuous-time Moran model, thus explicitly representing the evolution of individual parasite genomes. Implemented as a simulation, we use the model to explore relationships between parasite genetic diversity and epidemiology. First, we explore how varying prevalence influences parasite genetic diversity at equilibrium. We find a variety of genetic diversity statistics are correlated with prevalence, but the strength of these relationships depends on whether variation in prevalence between regions is driven by host- or vector-related interventions. Next, we explore the responsiveness of a variety of statistics to control interventions, and find that those related to mixed infections respond quickly (~months) whereas other statistics, such as nucleotide diversity, may take several years to respond. Ultimately, we expect these results will help guide efforts to monitor malaria epidemiology using genetic data.

244

DETERMINING THE KEY FACTORS THAT IMPACT THE PROGRAMMATIC PERFORMANCE OF SEASONAL MALARIA CHEMOPREVENTION: A MODELING APPROACH

Kamaldeen Olatunde Okuneye, Jaline Gerardin

Institute for Global Health, Northwestern University, Chicago, IL, United States

Since its recommendation by the WHO in 2012, Seasonal Malaria Chemoprevention (SMC) with sulfadoxine-pyrimethamine plus amodiaquine (SP+AQ) has been effective in protecting children under 5 years old from infection in the Sahel, where malaria transmission is highly seasonal. This study uses mathematical modeling of malaria transmission dynamics, pharmacokinetics and pharmacodynamics of SP and AQ, and SMC campaigns to compare how various factors such as coverage level, SMC timing, drug adherence, and partial dosing affects the programmatic performance of SMC. We calibrated the model against parasitemia prevalence and reduction in clinical cases from a non-randomized SMC trial in Kita, Mali, conducted in August through November of 2014. We modeled the coverage of SMC by considering a semi-correlated coverage, where the total population of children under 5 years old are divided into two groups of high and low accessibility to SMC. Children in the high-access group receive all four rounds of SMC, and those in the low access-group have a 35% chance of receiving each round of SMC. This pattern of semi-correlated coverage was observed in the programmatic deployment of SMC in Kita with an overall coverage of 69.7%. We show that this SMC coverage scenario provides a similar impact as uncorrelated coverage on cases averted. We predict that increasing the overall coverage of SMC in Kita from 69.7% to 87.5% would increase the reduction in clinical cases from 68% to 86.7%. Furthermore, we find that in highly seasonal settings, even two weeks can make a large difference in SMC impact: the model predicted that starting SMC on August 1st in Kita reduces clinical incidence by 68.5% compared to only 40.3% when the SMC campaign begins on August 14th. The model further predicts that adherence to amodiaquine on days 2 and 3 of the monthly round does not impact the effect size of SMC. Overall, we suggest that the decay in SMC impact when in programmatic deployment is more likely to be due to insufficient coverage and suboptimal timing rather than poor adherence, or incomplete swallowing of the entire dose.

245

ASSESSING HEALTH SYSTEM FACTORS INFLUENCING THE PREVENTION OF MALARIA AMONG PREGNANT WOMEN IN GHANA

Virtue Fiawokome De-Gaulle[1], Pascal Magnussen[2], Harry Tagbor[3]

[1]*University Of Yaounde 1, Yaounde, Cameroon,* [2]*University Of Copenhagen, Copenhagen, Denmark,* [3]*University Of Health and Allied Sciebces, Ho, Ghana*

Malaria interventions including use of Sulphadoxine-Pyrimethamine as Intermittent Preventive Treatment (IPTp-SP) and distribution of Insecticide Treated Nets (ITNs) have been implemented through ante-natal clinic (ANC) services. Yet, the high ANC attendance is not commensurate with the uptake of these interventions in Ghana. This study sought to assess the health system factors influencing the implementation of the interventions to prevent malaria among pregnant women in the Volta region of Ghana. A cross-sectional study was conducted in seven health facilities across two districts in the Volta region. Employing a mixed-concurrent method, questionnaires, structured observation checklists, and in-depth interview guides were used. Data collection entailed non-participant observation of ANC sessions, followed by exit interviews with pregnant woman and in-depth interviews with key personnel at the district, regional and national levels. Delivery of IPTp-SP and ITN were assessed using a defined cumulative effectiveness algorithm and predictors of effective delivery was determined using bivariate and multivariate logistic regression. Thematic content analysis was employed for the qualitative data analysis. Approximately 97% of the total (680) ANC observations had complete information for analysis. Of these, about 16% (103) were in their first trimester and 42% were in their second (279) and third (275) trimesters. 509 were eligible for IPTp-SP, of which 77% (391) had IPTp-SP delivered effectively. ITN was effectively delivered to 84% of the observed ANC registrants (n=127) with stock out at the facility being a major factor affecting effective delivery. At the facility level, qualitative findings revealed that incorrect interpretation of policy on eligibility for IPTp-SP accounted for ineffective delivery. At the national level, delay in procurement of SP and quality checks accounted for stock out. In conclusion, stock out of SP were due to national procurement challenges with missed opportunities for uptake due to incorrect knowledge of health workers on pregnant woman's eligibility for IPTp-SP.

246

DETERMINING BARRIERS AFFECTING MALARIA CASE MANAGEMENT SERVICES UPTAKE IN LIBERIA 2019

Victor S. Koko[1], Wahdae-mai Harmon-Gray[2], Eric Diboulo[3], Levi Hinneh[1], Joseph Alade[1], Jamesetta Smith[1], Julius Gilayeneh[1], Oliver Pratt[1]

[1]*Ministry of Health/National Malaria Control Program, Monrovia, Liberia,* [2]*Technical Assistant, Monrovia, Liberia,* [3]*MEASURE Evaluation, Monrovia, Liberia*

Malaria causes millions of illnesses globally with the highest-burden in Sub-Saharan Africa (95%). In Liberia, malaria accounts for 34% of outpatients' attendance and22% inpatients' deaths and has a prevalence of 45% using mRDT. Since 2016, malaria-related morbidity decreased by 57% per 100,000 persons and under-five mortality reduced by 64% from 116 per 100,000 in 2013 to 42 per 100,000 in 2018.Household nets ownership increased from 50% to 62% and utilization remains low (32%-39%); ANC attendance at 55% andIPT3+ uptake (22%). This progress is a foot into the future but slow for the desired impact to attain greater alignment to national and global targets towards pre-elimination. Despite increased investments and interventions coverage, uptake of services remains relatively low thus creating fear of not achieving the goal of reducing morbidity and mortality by 50%by 2020. This study employed a mixed-method approach (Qualitative and Quantitative) and was conducted in six (6) counties. Data collection Included patient exit interview, Key-informant interview (KII), community focus groups discussions (FGDs)and

drugs and data audit. Stock-out of commodities was a cross cutting barrier identified by both health workers and community members. The study found limited training of health workers in malaria case management, low salary and under-staffing of facilities as barriers. At community level, cost, access, health workers attitude, and waiting time were found to be barriers affecting malaria case management services. ACTs were stock-out at 44% of facilities visited while 55% and 45% were stock-out of Artesunate IM/IV and Artemether IM respectively. Ten (12%) of 83 patients were diagnosed without laboratory confirmation contrary to the guidelines with few patients given prescriptions (due to stock out). Additionally, the study found inconsistent data between the data source (health facility ledgers) and health management information system (HMIS) thus pointing to poor data quality. In light of these, there is a need for strengthening both human and material capacities of the health system to address these barriers

247

A HISTORIC MOVE IN CAMBODIA TO TREAT *PLASMODIUM VIVAX* MALARIA WITH 14-DAY TREATMENT OF PRIMAQUINE WITH G6PD TESTING

Sokomar Nguon[1], Sharon Thangadurai[1], Ranjith De Alwis[1], Paul Bacon[1], Samphornarann Top[1], Linna Khorn[1], Sam A. Sen[1], Soriya Samrith[1], Sidavy Kun[1], Vanna Oum[1], Sanann Nou[1], Rekol Huy[2], Dysoley Lek[2], Sovannaroth Siv[2], Bunreth Voeurng[3], Davoeurng Chan[3], Neang Ang[4], Sovann Yok[4], Sarith Oum[4]

[1]*University Research Co., LLC, Phnom Penh, Cambodia,* [2]*National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia,* [3]*Provincial Health Department of Battambang, Battambang, Cambodia,* [4]*Provincial Health Department of Pailin, Pailin, Cambodia*

The 8-aminoquinoline drugs, primaquine (PQ) and tafenoquine, are currently the only effective options to treat *Plasmodium vivax* (Pv) and prevent relapses of the persistent liver stage of the parasite. However, a major side effect of these drugs is the potential for severe hemolysis in people with glucose-6-phosphate dehydrogenase (G6PD) deficiency. In Cambodia, where the prevalence of G6PD deficiency is high (2% at the East to 14% at the est), radical cure using these drugs is very carefully administered and only allowed after G6PD testing. In 2019-2020, the Cambodia National Malaria Control Program (CNM) implemented PQ for radical cure of Pv and mixed (Pv and *Plasmodium falciparum*) malaria infections in four of 25 provinces in Cambodia (Battambang, Kampong Chhnang, Kampong Speu, and Pailin). University Research Company (URC), through the USAID/PMI Cambodia Malaria Elimination Project (CMEP) has supported implementation of radical cure for Pv in Battambang and Pailin provinces. After completing training and ensuring all supplies of G6PD Rapid Diagnostic Tests (RDT) and PQ (15 mg & 7.5 mg tablet) were in place, CMEP initiated G6PD testing and radical treatment with 14 days PQ administration for males weighing at least 20 kg diagnosed with Pv in Battambang and Pailin provinces. As of January 31, 2020, 67 Pv cases were enrolled and 61 G6PD RDTs were used to test 53 patients (some retested and some not eligible). Of the 53 cases tested, 52 had normal G6PD levels and one was G6PD deficient. All 53 cases received artesunate + mefloquine (ASMQ) treatment for 3 days as directly observed therapy while the 52 cases with normal G6PD levels received an additional 14 days of PQ treatment. The 52 cases with normal G6PD were also visited on days 7 and day 14 to ensure compliance with PQ administration, in addition to checking if any case experienced adverse reactions. Of the 52 cases receiving PQ treatment, 3 day, 7 day and 14 day follow up was completed. No adverse reactions were reported. These results provide support and reassurance of CNM's plans to scale up Pv radical treatment with PQ along with G6PD testing and 14-day follow-up country wide.

248

FOREST MALARIA INTERVENTIONS HAVE MADE REMARKABLE PROGRESS IN DRASTICALLY REDUCING *PLASMODIUM FALCIPARUM* MALARIA IN PURSAT PROVINCE, CAMBODIA

Sharon Thangadurai[1], Sokomar Nguon[1], Ranjith De Alwis[1], Paul Bacon[1], Samphornarann Top[1], Linna Khorn[1], Siv Krung[1], Say Mang[1], Kongmeng Seak[1], Phally Chhun[1], Rekol Huy[2], Thavrin Boukheng[2], Sovannaroth Siv[2], Dy Khoy[3], Kimen Ke[3], Tino Mey[3], Sokha Ratha[3], Sovannak Kong[3], Dong Phe[3]

[1]*University Research Co., LLC, Phnom Penh, Cambodia,* [2]*National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia,* [3]*Provincial Health Department of Pursat, Pursat, Cambodia*

Malaria risk is highest in forest and forest fringe areas of Cambodia and is linked to the movement of mobile and migrant populations (MMP) visiting the forest for economic reasons. In 2018, the Cambodia National Malaria Program (CNM) introduced a robust intensification plan (IP) in the 10 malaria endemic Operational Districts (OD). All key stakeholders, analyzed and identified 29 villages, 6 health facilities, and numerous hotspots in Pursat province to implement IP. CMEP supported CNM to equip and train mobile malaria workers (MMWs) identified among the most at risk populations in two of the ODs (Phnom Kravanh and Krakor) to proactively provide point-of-care early diagnosis and treatment. CMEP also provided the MMPs with insecticide-treated mosquito nets (ITNs) and educational materials. As of 2019, 42 MMWs (called fixed touch points and forest goer peer volunteers) were trained to carry out malaria activities. These MMWs reached out to forest goers/workers, tested and treated them, and educated them on prevention and care seeking. The MMWs were provided with on-the-job training and supervised by public health staff. Results showed a 57% decline in overall malaria cases (14,483 cases in 2018 to 6,184 cases in 2019), including a 82% decline for *Plasmodium falciparum* (Pf) cases (3,941 cases in 2018 to 696 cases in 2019). Screening and testing increased more gradually, suggesting that malaria parasite reservoirs were interrupted, limiting any new malaria transmission. Annual parasite incidence (API) dropped from 78/1,000 population (Pf: 21.2/1,000) in 2018 to 32.7/1,000 population (Pf: 3.7/1,000) in 2019 while test positivity rate (TPR) dropped from 40% in 2018 to only 11% in 2019, along with an increase in annual blood examination rate (ABER) from 19% to 29%. Expansion of malaria services in hotspots through MMWs' proactive case detection (Pro-ACD) improves the effectiveness of reducing malaria incidence. Routine or passive case detection at public health facilities still continues to play an effective role. Moving towards sustainability the national program could integrate this as part of the routine services.

249

THE IMPACT OF DIET ON MALARIA SEVERITY IS DIFFERENTIALLY MODULATED BY THE GUT MICROBIOTA

Kristin M. Van Den Ham, Nathan W. Schmidt

Indiana University School of Medicine, Indianapolis, IN, United States

Diet plays an integral role in shaping the structure and function of the microbial community in the gastrointestinal tract. Many studies have examined the influence of dietary ingredients on the gut microbiome; however, these studies have often focused on the impact of extreme, supraphysiological changes to the macronutrient content of diets and their subsequent dramatic transformations of the gut microbiota. The few studies that have examined the impact of more moderate diet modifications have concluded that these alterations result in negligible or only minimal changes to the composition of the murine gut microbiota. Interestingly, we observed a significant difference in the ability of mice to control *Plasmodium yoelii* infection that was entirely contingent on which diet they were fed. The diets provided to the mice had a very similar macronutrient profile and the variation in their micronutrient and ingredient composition both fell under the purview of a "standard" mouse diet. Importantly, the influence of diet on the ability of mice to repress

P. yoelii parasitemia appeared to be dependent on their respective gut microbiotas, as the impact of diet correlated with the vendor source of the mice, which we have previously shown to correspond to disparate gut microbial communities. To investigate the interrelationship between diet, gut microbiota and immune response, differences in the micronutrient and ingredient composition were identified and diets were created to unravel the specific nutrients and non-nutritive factors responsible for the difference in malaria susceptibility. Conceivably, these experiments will allow us to identify synergistic pairings of probiotics and prebiotics (synbiotics) that have the capacity to manipulate the structure or metabolism of the gut microbiota and consequently influence the severity of malaria.

250

INVESTIGATION OF OUTBREAK OF MALARIA AND OTHER FEBRILE ILLNESSES IN A REMOTE SEMI-ARID COUNTY, KENYA, 2019

Josphine Waihuini Ihahi, Elvis Oyugi, Maurice Owiny, Joseph Ogutu, Hosea Serech

Field epidemiology and laboratory training program (FELTP), Nairobi, Kenya

Malaria accounts for 5.6% of mortalities in Kenya annually with seasonal epidemics in arid regions. An outbreak of malaria and other febrile illnesses was reported in semi-arid Baringo County in September, 2019. We sought to investigate the outbreak and institute control measures. We reviewed records in health facilities, conducted active case search (ACS), administered structured questionnaires to cases defined as any patient presenting with fever with/without chills, headache, or joint pains from July–August, 2019. Whole blood samples were collected for mRDT and microscopy at two laboratory tiers; County laboratories (CLs) and national reference laboratories (NRLs). Kappa statistics was used to compare microscopic readings. PCR, serological and molecular assays were conducted on serum to identify other febrile illnesses. We conducted community outreaches with targeted interventions. We identified 1,224 cases, eight deaths (CFR=0.6%). Females were 697/1224 (56.1%). ACS yielded 1,109/1,224 (89.3%) cases; 199/1,109 (17.9%) through community outreaches. Overall attack rate was 1224/180,766 (0.7%) and 424/29,778 (1.4%) for children aged <5years. Epidemic curve depicted intermittent transmission. Of 72/199 (36.2%) cases interviewed; 65/72(90.3%) had fever, headache 47/72 (65.3 %) and chills 37/72 (51.4%). Of 63 samples analysed: 40 (63.5%) were reactive for mRDT with 93% sensitivity and 63.6% specificity against NRLs microscopic readings. *Plasmodium falciparum* was identified in 28/63(44%, 95% CI 31.9-57.5) in CLs and 30/63(48%, 95% CI 34.9-60.6) in NRLs (Kappa agreement value of 90.3%). On serological and molecular assays; 17/60 (28%) samples were reactive for anti-DENV IgM, 32/60 (53 %) for anti-CHIKV IgM and 13/60 (22%) for anti-DENV IgG, 7/30 (23%) malaria positive samples were anti-DENV IgM reactive, 14/30 (46.7 %) for anti-CHIKV IgM and 1/30(3.3%) for anti-DENV IgM and anti-CHIKV IgM. Forty (40) households were reached with health education and malaria control interventions. *P. falciparum* was identified as the cause of malaria. Other febrile illnesses included chikungunya and dengue fever.

251

SCOPING REVIEW OF THE KEY DETERMINANTS AND INDICATORS OF MALARIA IN PREGNANCY, MADAGASCAR (2010-2019)

Catherine Dentinger[1], Natasha Hansen[2], Susan Youll[2], Annett Cotte[1], Mary Lindsay[3], Chiarella Matten[4], Vololoniala Aimee Ravaoarinosy[5]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*US Agency for International Development, Washington DC, DC, United States,* [3]*US Agency for International Development, Washington, DC, United States,* [4]*Institut Pasteur de Madagascar, Antananarivo, Madagascar,* [5]*National Malaria Control Program, Antananarivo, Madagascar*

Malaria in pregnancy (MIP) increases the risk of poor maternal and infant outcomes; to prevent this, the World Health Organization (WHO) recommends insecticide-treated net (ITN) use, intermittent preventive treatment during pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP), and prompt case management. In Madagascar, IPTp uptake remains low; 10% of targeted women receive 3 doses. To determine if additional data are needed to improve MIP activities, we conducted a scoping review to identify barriers to antenatal care (ANC) and IPTp uptake. We searched PubMed, Google Scholar and USAID's files (Development Experience Catalog) using the terms "Madagascar" and "pregnancy" and "malaria" and collected materials from stakeholders. We included English and French documents from 2010 to 2019 with quantitative or qualitative data regarding malaria during pregnancy. Documents were reviewed and categorized as MIP background information, care seeking, and facility readiness. Of 69 project reports, surveys and published articles, 15 (22%) met the inclusion criteria; 4 (27%) were categorized as care seeking, 4 (27%) as background, and 7 (47%) as facility readiness. Eight (53%) articles mentioned SP stock outs, 3 (20%) mentioned poor provider knowledge of IPTp guidelines despite recent training, and 5 (33%) discussed barriers to ANC including distance, wait times, poor service quality, cost, and unfriendly providers. One study found only 30% of targeted health workers received recommended supervision. A 2015 survey of 52 health facilities revealed limited access to ANC due to financial and geographic barriers; 2018 surveys revealed similar findings. Self-treatment and care-seeking delays were reported even when distance was not a barrier. Our review revealed well-documented barriers to MIP services that could be mitigated by reducing stock outs, improving access to healthcare by removing fees and providing services closer to women's homes, and targeted behavior change. These findings can be used to guide coordinated donor and government efforts to address management, financial, and human resource gaps to improve MIP services.

252

IMPROVING ADHERENCE TO TEST-BASED MANAGEMENT OF UNCOMPLICATED MALARIA AMONG CAREGIVERS OF CHILDREN AND PRIVATE MEDICINE RETAILERS WITHIN RURAL COMMUNITIES OF FANTEAKWA NORTH DISTRICT, GHANA

Olajoju Temidayo Soniran, Benjamin Abuaku, Collins Ahorlu

Noguchi Memorial Institute for Medical Research, Accra, Ghana

Prompt diagnosis and treatment of malaria prevents a mild case from developing into severe disease and death. Unfortunately, presumptive treatment of malaria is prevalent among caregivers of febrile children and private medicine retailers that serve as their first point of call. A mixed method was used in this baseline study to determine factors limiting test-based management of suspected malaria cases among caregivers of febrile children and over the counter medicine sellers (OTCMS) in some rural communities of Ghana. Structured questionnaires were used to interview 254 adult caregivers. Fourteen in-depth interviews of OTCMS were conducted, audio-recorded, transcribed verbatim, and analysed thematically. Data were analysed using SPSS and N-Vivo. Malaria parasitological testing rate of febrile children is significantly associated with type of healthcare provider, highest (94.9%) at the government health facility and lowest (10.5%) at the OTCMS shops. Proportion of febrile children not subjected to malaria blood test is below average (28.3%). Some caregivers (47.8%) couldn't give a specific reason for their presumptive approach, 21.7% were financially handicapped to visit the health centre, 15.2% lacked knowledge of malaria blood test, while others (15.2%) gave reasons related to OTCMS. From OTCMS point of view, clients' inability to pay for malaria blood test, community perception that OTCMS are unqualified to perform malaria blood test, clients' adherence to presumptive treatment, financial loss when unused RDT kits expires, clients' demand for half dose of ACT, and activities of drug peddlers are

factors limiting adherence to WHO recommended policy on management of uncomplicated malaria. Barriers to test-based management of malaria include caregivers' ignorance, cost of seeking care, client-provider interaction, and pressure to heed clients' demand. Hence, we have commenced an interventional study aimed at improving universal access to quality diagnosis and treatment of suspected malaria cases through the OTCMS using interventions that are sustainable and scalable.

253

LONGITUDINAL ASSESSMENT OF INFECTIVITY OF *PLASMODIUM FALCIPARUM* INFECTIONS IN A LOW-MODERATE TRANSMISSION SETTING IN THE GAMBIA

Marta Moreno[1], Abdullahi Ahmad[2], Harouna M. Soumare[2], Muhammed M. Camara[2], Lamin Jadama[2], Mamadou-Ousmane Ndiath[2], Lamin Camara[2], Sainabou Drammeh[2], Blessed Etoketim[2], Thomas Mendy[2], John Bradley[1], Jane Achan[2], Teun Bousema[3], Umberto D'Alessandro[2], Chris Drakeley[1]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*Medical Research Council Unit The Gambia at London School of Hygiene & Tropical Medicine, Fajara, Gambia,* [3]*Radboud Institute for Health Sciences, Nijmegen, Netherlands*

To support malaria elimination efforts, it is critical to better understand the contribution of clinical and subclinical *Plasmodium falciparum* infections to malaria transmission. We longitudinally measured parasite carriage, gametocyte carriage and infectivity to mosquitoes in eight villages (N= 2,375) with low-moderate endemicity (1.2-15.4% qPCR prevalence at the start of the transmission season) in Upper River Region, The Gambia. Four cross-sectional surveys were conducted during the 2019-2020 transmission season and Community Case Management of Malaria (CCM) by trained village health workers (VHW) was implemented to detect clinical malaria cases. Among the 176 *P. falciparum* clinical cases (median age of 12 years, IQR:7-19) identified by VHW, infectivity was assessed in forty-seven direct membrane feeding assays (DMFAs) involving 4613 dissected *Anopheles coluzzii* mosquitoes [74-115 mosquitoes per participant]; none of which became infected. Asymptomatic *P. falciparum* infections were identified during cross-sectional surveys by *var*ATS-qPCR. Parasite prevalence (≥ 0.1 parasites/ µL) in cross-sectional surveys ranged between 2.8% and 24.2%: 61% of infections had <1 parasite/µL and only 10.5% over 1,000 parasite/ µL. From these surveys, 103 participants were recruited for DMFA and eight subjects (7.7%; 8/103) infected 9% of 10 341 mosquitoes dissected [74-118 per feeding assay] with 1-25 oocyst per midgut. Children aged 5-15 years (50%) and adults (50%) were responsible for all the mosquito infections. We conclude that in this area of low-moderate transmission intensity with an average clinical malaria incidence of 0.05 episodes/ person/season, where CCM is implemented, the vast majority of mosquito infections arise from asymptomatic parasite carriers. In this study, the majority of asymptomatic infections persist at densities too low to allow onward transmission to mosquitoes.

254

DETERMINANTS OF IMPROVEMENTS IN HEALTH WORKERS' COMPLIANCE WITH OUTPATIENT MALARIA CASE-MANAGEMENT GUIDELINES IN KENYA

Beatrice Indimuli Amboko[1], Kasia Stepniewska[2], Philip Bejon[1], Robert W. Snow[1], Dejan Zurovac[1]

[1]*KEMRI-Wellcome Trust Research Programme, Nairobi, Kenya,* [2]*WorldWide Antimalarial Resistance Network, Oxford, United Kingdom*

Health workers' compliance with the outpatient malaria "test" and "treat" guidelines has been improving in Africa. We examined what factors are associated with the improvements. Secondary analysis of data from 11 national cross-sectional health facility surveys undertaken between 2010 and 2016 was undertaken. Association between 36 factors and improvements in compliance with four malaria "test" and "treat" indicators and administration of the first artemether-lumefantrine (AL) dose at the facility were examined using multilevel logistic regression models. Improvement in the overall health worker's compliance was associated with: lake endemic compared to coast endemic (aOR=1.42), highland epidemic (aOR=1.23), semi-arid seasonal transmission (aOR=1.71) and low risk (aOR=1.67) zones; availability of rapid diagnostic tests (RDTs) compared to either both microscopy and RDTs (aOR=1.58; 95% CI 1.31-1.90) or microscopy only (aOR=1.49; 95% CI 1.28-1.73); caseload of >25 patients (aOR=1.46; 95% CI 1.14-1.87); faith based organization (FBO) compared to government facilities (aOR=1.15; 95% CI 1.01-1.30) and under-fives compared to older patients (aOR=1.07; 95% CI 1.02-0.1.14). Other factors associated with improvements in the specific components of the "test" and "treat" policy and AL administration included; absence of RDTs stock-outs, cadre dispensing drugs at the facility, health workers' access to malaria case-management and IMCI guidelines, health workers' gender, correct knowledge about the malaria treatment policy, and patients' main complaint of fever. In-service training and supervision were not associated with improvements. Malaria control programs should; ensure continued availability and provision of RDTs in facilities without diagnostic capabilities, improve health workers' knowledge about the policy emphasizing on age and fever, and disseminate malaria and IMCI guidelines. Targeting of male, government health workers and low malaria transmission areas is recommended. For administration of first AL dose at the facility, task-shifting of duties to community health workers can be considered.

255

IMPROVEMENT IN MALARIA DATA QUALITY AND USE IN THE PRESIDENT'S MALARIA INITIATIVE SUPPORTED PROVINCES IN THE DEMOCRATIC REPUBLIC OF CONGO FROM 2015 TO 2019

Johanna Karemere[1], Jean Marie Ngbichi[1], Erick Katanga[2], Hyacinthe Kaseya[3], Olivier Kakesa[1], Yung-Ting Bonnefant[4], Aboubacar Sadou[4]

[1]*ICF / PMI Measure Malaria, Kinshasa, Democratic Republic of the Congo,* [2]*Direction de Système National d'Information Sanitaire (DSNIS):RDC, Kinshasa, Democratic Republic of the Congo,* [3]*National Malaria Control Program (NMCP)/ DRC, Kinshasa, Democratic Republic of the Congo,* [4]*PMI, Kinshasa, Democratic Republic of the Congo*

The National Malaria Control Program (NMCP) in the Democratic Republic of Congo (DRC) needs quality routine malaria data to better inform program implementation. The purpose of this research is to determine whether technical and institutional support provided by the USAID-funded MEASURE Evaluation project to the DRC health information systems (HIS) and NMCP improved the quality and use of malaria data. Key project activities were reviewed and HIS indicators compared over time. In 2014, the DRC HIS and NMCP faced major data challenges, without a functional District Health Information Software (DHIS2) platform, data collection tools, or M&E guiding manuals. Based upon an M&E capacity assessment, the project strengthened the NMCP and sub-national health levels to collect, report, analyze and use routine malaria data in the nine provinces supported by the US President's Malaria Initiative (PMI). The project set up a surveillance, monitoring and evaluation technical working group (SMETWG) to strengthen the NMCP and provincial levels; contributed to the roll-out of the DHIS2 platform by providing computers and internet connection to 77 health facilities; supported the integration of malaria indicators into the DHIS2; and supplied data collection and reporting tools. Additionally, the project trained 400 NMCP and sub-national health providers on M&E fundamentals and provided technical support for data management, data quality audits, data analysis and use. All 178 supported health zones held monthly malaria data analysis and validation meetings, the SMETWG met quarterly, and quarterly routine data quality assessments were conducted. These efforts improved the validity and consistency of malaria data generated through the HIS. Additionally, the monthly data review meetings fostered improved knowledge and use of data through discussion and feedback. Completeness of reporting in the nine PMI-supported provinces increased from 0.1% in 2015 to 97.2% in 2019, and

timeliness went from *"no data"* in 2015 to 68.6% in 2019. PMI support to DRC has improved malaria data in nine provinces and has had an overall positive impact on the health system in DRC.

256

PERFORMANCE OF ELECTRONIC DISEASE SURVEILLANCE SYSTEM IN MADAGASCAR: EVIDENCE FROM COMPARATIVE STUDY AMONG TWO CLUSTERS OF HEALTH DISTRICTS

Maurice Ye[1], Lea Bricette Randriamampionona[2], Jean-Marie N'Gbichi[3], Laurent Kapesa[4], Jocelyn Razafindrakoto[4], Armand Solofoniaina Rafalimanantsoa[2], Mauricette Nambinisoa Andriamananjara[5], Yazoume Ye[3]

[1]*PMI Measure Malaria, ICF Macro Madagascar, Antananarivo, Madagascar,* [2]*Department of Epidemiological Surveillance and Response, Ministry of Public Health, Antananarivo, Madagascar,* [3]*PMI Measure Malaria, ICF, Rockville, MD, United States,* [4]*President's Malaria Initiative, Health Population and Nutrition Office, Antananarivo, Madagascar,* [5]*National Malaria Control Program, Ministry of Public Health, Antananarivo, Madagascar*

The use of an electronic disease surveillance system (ESS) has the potential to optimize infectious diseases detection and response. ESS involves the use of electronic devices (tablets, smartphones, etc.) to collect and report real-time data for decision-making. In 2016 Madagascar introduced ESS in parallel with paper-based reporting (PBR) to track the 28 priority diseases including malaria. ESS was implemented in 1,182 *Centre de Santé de bases (CSB)* in 58 of 114 districts reporting weekly to central level. To inform country-wide scale-up of ESS, an assessment was conducted in 2019 to assess the performance compared to PBR and document potential technological challenges. The assessment method included desk review and secondary data analysis of data extracted from reports. Two indicators were assessed: completeness and timeliness of reports. Completeness was defined as number of surveillance reports submitted during the reporting period out of total number expected (target:90-100%). Timeliness was defined as number of reports submitted on time out of total number reports expected (target 85-100%). We used two-sample test of proportions (Z-test), with significance level of P-value<0.05, to compare the performance of reporting between 58 districts with ESS and 56 districts with PBR. Overall, districts with ESS reported (70%) completeness against (77%) for districts using PBR. However, the difference was not statistically significant (p-value=0.397). Similarly, timeliness reporting was statistically similar in both groups of districts (ESS=51%, PBR =47%, p-value=0.669). In the context of recurrent epidemics in the country (plague, measles, etc.) the system was determinant to deliver real-time 100% of alerts coming from CSB. Regarding the status of the equipment, overall 953 tablets out of 1,238 (77.0%) were functional and 258 tablets (20.8%) damaged. Major challenges encountered with ESS were weak robustness of tablets, slow internet connectivity, low workers skills in using tablets, irregular supervision, and poor system maintenance. These challenges should be addressed before scaling-up the system.

257

UGANDAN MALARIA CASE MANAGEMENT PRACTICES BY HEALTH WORKERS, ASPECTS OF IMPROVEMENT AND RESILIENCE

Patricia Mukose

Malaria Consortium, Kampala, Uganda

The US President's Malaria Initiative (PMI) supported Malaria Action Program for Districts (MAPD) project supports malaria control in Uganda to building health worker (HW) capacity. We assessed HW practices in 9 MAPD districts and compared them to areas in other places and assessed HW practices during periods of malaria upsurge in both intervention and control areas. Health facility (HF) data between January 2017 and December 2019 was grouped into 3 periods; Pre - intervention (pre-int), intervention with no malaria upsurge (Int-no upsurge) and intervention with malaria upsurge (Int-upsurge). Three outcomes were assessed: proportion of patients suspected to have malaria and proportion of those with a negative test result but treated for malaria, and proportion of malaria-related deaths. In intervention area, 82% suspected to have malaria in the pre-int period were tested, increasing to 92% (Int-no upsurge), and to 94% (Int-upsurge). In comparison area this was 65% in both pre-int and Int-no upsurge periods, 37% during Int-post upsurge. The proportion of those testing negative but treated in MAPD areas decreased from 34% (pre-int) to 21% (Int-no upsurge) to 4% (Int-post upsurge), whereas in comparison area this reduced from 48% to 17%, but rose to 32% during upsurge. The proportion of malaria deaths reduced from 18.4% (pre-int), to 3.1% Int-no upsurge) and to 1.8% (Int-upsurge) in intervention areas but increased from 1.7% to 3.2% in control areas

258

THE EPIDEMIOLOGICAL AND PROJECTED ECONOMICAL IMPACT OF INDOOR RESIDUAL SPRAYING IN NGOMA DISTRICT, RWANDA

Michee S. Kabera[1], Noella Noella[2], Emmanuel Hakizimana[1], Dunia Munyakanage[1], Jean Louis Mangala[1], Kaendi Munguti[3], Aimable Mbituyumuremyi[1]

[1]*Rwanda Biomedical Center, Kigali, Rwanda,* [2]*JHPIEGO, Kigali, Rwanda,* [3]*PMI, Kigali, Rwanda*

Malaria remains a major public health problem and is among the leading causes of morbidity and mortality in Rwanda. Indoor Residual Spraying (IRS) is a core vector control intervention in Rwanda that supplement Insecticide-treated bed nets (ITNs). Ngoma district is located in Eastern province of Rwanda bordering with Burundi in South. The first IRS round was conducted in the above district in April 2019 (before the high peak season of May-June) with the support of the Government of Rwanda and The Global Funds. The IRS coverage rate was 98.9% with a protected population of 357,058 using "Pirimiphos methyl 300 CS. Using RHMIS, we compared malaria cases respectively reported for a period of 12 months before IRS (April 2018 to March 2019) and after IRS (April 2019 to March 2020). The total number of uncomplicated malaria cases was significantly dropped down by 82%, from 581,742 before IRS to 105,120 cases after IRS. The incidence per 1000 inhabitants decreased from 1,502 to 265 respectively. Moreover, the inpatient cases also significantly decreased by 87.7%, from 1037 before IRS to 170 cases after IRS. The cost of conducting IRS in Ngoma district was USD 2,104,007 including both the cost for Insecticides and operation. The invested cost per averted malaria case was USD 4.4. Using the minimum average cost of USD 8.6 for treating an episode of the disease including direct cost and the opportunity costs of travel and waiting time [(1,2)]; the total benefit due to averted malaria cases is estimated to USD 4,132,313. Applying the average cost for inpatient malaria case which is estimated to USD US$60.44 [(4)], the benefit due to the averted malaria inpatient cases is equal to USD 52,401. The total benefit for averted outpatients and inpatients is estimated to USD 4,184,714. In conclusion, there was a significant decrease of out and inpatient malaria cases just one year after IRS in Ngoma district. Furthermore, if we compare the IRS expenditures and the benefits related to the averted malaria cases, there was an important cost benefit. We expect more economic impact as malaria cases may continue to decrease.

260

ASSESSMENT OF MALARIA CASE MANAGEMENT PRACTICES AMONG HEALTH WORKERS IN KANO AND ZAMFARA STATES, NIGERIA: RESULTS OF THE MALARIA FRONTLINE PROJECT; 2016-2019

Adefisoye O. Adewole[1], Usaini Bala[1], Amina Umar Abdullahi[1], Ndadilnasiya Waziri[1], Saheed Gidado[1], Gideon Ugbenyo[1], Edwin Simple[1], Adam Attahiru[1], Shelby Cash[2], John Williamson[2], Kwame Asamoa[2]

[1]*African Field Epidemiology Network, Abuja, Nigeria,* [2]*Malaria Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States*

The Malaria Frontline Project (MFP) is a 3-year CDC collaboration with AFENET and National Malaria Elimination Program in Kano and Zamfara States, Nigeria. Gaps in healthcare workers (HCWs) malaria case management practices identified during baseline assessment were addressed by training HCWs with materials well-tailored to their service delivery, post-training exercises and supportive supervision. We assessed HCW case management practices at the end of the project. Twenty-four primary healthcare centres [PHCs] in Kano and 24 PHCs in Zamfara were sampled. A semi-structured questionnaire was administered to two HCWs purposively selected from each PHC. Their practices were verified through five consecutive febrile client exit interviews in each selected PHC. Comparative analysis was done between baseline and end-line assessments at 95% confidence interval [C.I] and $p<0.05$ using Statistical Package for Social Sciences version 20 software. A total of, 180 HCWs (Kano:100; Zamfara:80) were interviewed during end-line compared to baseline 158 HCWs (Kano:83; Zamfara:75). HCWs' adherence to national guidelines on malaria diagnosis, baseline and at end-line: Kano (90.4% [95% C.I:84.1-97.3] to 97.0% [93.7-100], p=0.97), Zamfara (90.7% [84.1-97.3] to 98.8% [96.4-100], 0.62]. HCWs' use of ACTs for malaria treatment, baseline and at end-line (Kano: 80.7% [72.2-89.2] to 99.0% [97.0-101.0], 0.62), Zamfara: (72.0% [61.8-82.2] to 81.3% [72.8-89.8], 0.01). Clinicians' adherence to national treatment guidelines, baseline and at end-line (Kano: 73.3% [63.3-83.3] to 97.7% [95.1-100.3], 0.87) and (Zamfara: 79.2% [67.7-90.7] to 96.6% [93.7-99.5], 0.03). Clients that received ACT, baseline and at end-line (Kano: 44% [28.2-60.6] to 69% [60.3-78.5], 0.08) and Zamfara: (68% [49.7-82.3] to 66% [56.7-74.3], <0.001). The MFP strategy of using tailored training materials, job aids and supportive supervision improved HCWs adherence to guidelines on malaria case management. This strategy may be adapted to improve malaria and similar health programs in other Nigeria States and in other malaria endemic countries.

261

FACTORS ASSOCIATED WITH LONG LASTING INSECTICIDAL NETS USE IN HOUSEHOLDS DETECTED WITH MALARIA CASES IN ZANZIBAR, 2012-2019

Humphrey Mkali[1], Shabbir Lalji[1], Abdul-wahid Al-mafazy[1], Joseph Joseph[1], Abdullah Ali[2], Faiza Abbas[2], Mohamed Ali[2], Wahida Hassan[2], Mwinyi Khamis[2], Chonge Kitojo[3], Naomi Serbantez[3], Erik Reaves[4], Bilali Kabula[1], Ssanyu Nyinondi[1], Donal Bisanzio[5], Mike McKay[5], Richard Reithinger[5], Jeremiah Ngondi[5]

[1]*RTI International, Dar Es Salaam, United Republic of Tanzania,* [2]*Zanzibar Malaria Elimination Programme, Zanzibar, United Republic of Tanzania,* [3]*US President's Malaria Initiative, United States Agency for International Development, Dar Es Salaam, United Republic of Tanzania,* [4]*Malaria Branch, Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, and US President's Malaria Initiative, Dar Es Salaam, United Republic of Tanzania,* [5]*RTI International, Washington DC, NC, United States*

Mosquito nets remains an important tool to reduce malaria risk at both the individual and community level in Zanzibar. However, the 2017 Malaria Indicator Survey (MIS) data indicates that Long-Lasting Insecticidal Net (LLIN) use, especially among children (67.2%) and pregnant women (63.4%) remains low. This study aimed to identify factors associated with LLIN use among persons in households in Zanzibar where malaria cases were detected. Data from the malaria case-based surveillance system were collected from August 2012 to December 2019. Net density was calculated by dividing the number of LLINs by the number of residents per household. LLIN use was defined as the proportion residents that slept under an LLIN the night before the visit. We conducted univariate and multivariate logistic regression analyses of the association between LLIN use and explanatory factors (age, sex, pregnancy, net density, indoor residual spraying and number of sleeping places). A total of 81,473 participants were included in the analysis, of which 53.9% (43,914) reported to have slept under a LLIN the previous night. The average number of residents per LLIN was 2.83 (95% CI 2.81-2.84), with highest [3.14 (95% CI 3.09-3.17)] in 2014 and lowest [2.39 (95% CI 2.36-2.42)] in 2017. Factors associated with decreased LLIN use included: age ≥ 5 years [OR=0.35; 95% CI 0.31-0.39]; males [OR=0.77; 95% CI 0.75-0.80], net density <0.5 [OR=0.24; 95% CI 0.16-0.35], net density 0.5-1.0 [OR=0.63; 95% CI 0.43-0.93], participants without LLINs [OR=0.03; 95% CI 0.02-0.04] and number of sleeping places in household [OR=0.94; 95% CI 0.93-0.95]. Pregnancy was associated with increased LLIN use [OR=1.12; 95% CI 1.03-1.22]. These findings suggest that improved access to LLINs is needed to achieve universal coverage above 80%. Improved behavior change communication targeting male and older household members could be conducted to re-emphasize LLIN use.

263

PREVALENCE AND DEMOGRAPHIC FACTORS ASSOCIATED WITH MALARIA INFECTION AMONG INDIVIDUAL ATTENDING THE SCREENING FOR THE FUTURE MALARIA CLINICAL TRIAL IN BIOKO ISLAND & EQUATORIAL GUINEA

Marta amelia Muakuku Mosanga[1], Vicente Urbano Nsue Ndong1[2], Maria Silvia Angue Lopez Mikue[2], Thabit Mbaga[3], Beltran Ekua Ntutumu[2], Ali Hamad[3], said Jongo[3], Wonder Philip Phiri[1], Juan Carlos Momo[1], Martin Eka[2], Genaro Nsue[2], Matilde Riloha Rivas[4], Raul Chuquiyauri[1], Preston Church[5], Maximillian Mpina[6], Elizabeth Nyakarungu[3], Peter Billingsley[5], Claudia Daubenberger[6], Thomas Richie[5], Julia Yumbe Baka[1], Ines Toichoa[1], Olivier Tresor[1], Stephen L. Hoffman[5], Godwin Fuseini[1], Salim Abdulla[3], Guillermo Garcia[1], Kamaka Ramadhani Kassim[3]

[1]*Medical Care Development International, Malabo, Equatorial Guinea,* [2]*Ministry of Health and Social Welfare, Malabo, Equatorial Guinea,* [3]*Ifakara Health Institute, Dar es salaam, United Republic of Tanzania,* [4]*Ministry of health and social welfare, Malabo, Equatorial Guinea,* [5]*Sanaria Inc, Rockville, MD, United States,* [6]*Swiss Tropical and Public Health Institute, Basel, Switzerland*

Malaria is a preventable and curable communicable disease caused by a parasite. World Health Organization (WHO) reported that there were 228 million cases of malaria occurred worldwide in 2018 and most of these cases (93%) occurred in Africa. In Bioko Island, Equatorial Guinea there is an intensive Malaria Control Program implemented since 2004. Despite the presence of these intensive control efforts, the prevalence in 2018, 12.5%, was not significantly different from the 14% estimate of 2012. There is a need to conduct the prevalence studies in order to continue monitoring the effectiveness of current malaria control strategies and provide updated information. This is because the prevalence studies have the ability to give insights into the transmission patterns in any given area. Therefore, the objective of the present study is to provide current information on the prevalence of malaria infection and how demographic factors influence this malaria prevalence among the selected population of Bioko Island, Equatorial Guinea. The data for this study will be extracted from the clinical dataset of the study entitled *Pilot Study to Optimize Recruitment and Screening Procedures for Future Clinical Trials and to Create a Registry of Potential Research Participants on Bioko Island, Equatorial Guinea*. The pilot study is expected to recruit 3500 healthy males and females aged 18 months to 50 years old living in selected areas of Bioko Island with high malaria transmission. A thick blood smear (TBS) procedure is used to identify the presence of malaria disease during the screening visit or when the participant has signs and symptoms of

malaria. Statistical analysis will be done using SPSS windows software. The prevalence of malaria will be expressed in terms of percentage. The Pearson Chi-square test will be performed to determine the association between the socio-demographic factors and the prevalence of malaria infection. The level of statistical significance is considered at a p-value of < 0.05. The study started in September 2019 and final monitored data is expected to be available in July 2020.

264

KNOWLEDGE RELATED TO MALARIA SYMPTOMS, TRANSMISSION AND PREVENTION PRACTICES IN FRANCOPHONE SUB-SAHARAN AFRICA IMMIGRANTS IN WESTERN CANADA

Taylor Hanna, Ali Ahmed, Ryland Petrick, Vincent Étienne, Mélanie Elhafid, Michael Hawkes, Srilata Ravi, **Sedami Gnidehou**

University of Alberta, Edmonton, AB, Canada

Imported malaria (IM) is a growing public health challenge in many countries like Canada where malaria is non-endemic. IM is associated with travels to Sub-Saharan Africa, Latin America and South-East Asia regions which are endemic for the disease. As in Europe and in the United States of America, Canada's reported malaria cases are densely concentrated within regions exhibiting steady growth in immigrants visiting friends and relatives in their country of origin. It has been noted that immigrants visiting Sub-Saharan Africa, the predominant region of malaria infections and deaths worldwide, constitute the main target in this category. In the past few decades, the Edmonton Metropolitan Area in Western Canada has been experiencing a significant increase in immigrants, mainly French-Speaking (francophone) immigrants, from Sub-Saharan Africa. These immigrants constitute the main focus of our community-based descriptive study. In this study, we assessed knowledge related to malaria symptoms, transmission and prevention practices in 382 francophone immigrants born in Sub-Saharan Africa and living in Edmonton during the period of the study. We showed that, overall, knowledge of malaria as a fatal disease and fever as a symptom of the disease was accurate in the study population. However, while 99% of respondents considered mosquito bites as a means of transmission, a large proportion (92%) incorrectly believed that drinking dirty water and living in a dirty environment caused malaria infections. Interestingly, we identified that only 31% of respondents had a French speaking family physician even if 84% of the participants had declared French as the language in which they were most at ease. We also demonstrated that 37% of respondents were inadequately prepared for travel. The perception of a healthcare professional encounter as futile and the false notion of a long term acquired immunity were cited as reasons for not having a pre-travel medical encounter. This study highlights important steps to consider toward the implementation of appropriate, sustainable, and effective malaria prevention in Canada.

265

ESTIMATED LIVES SAVED IN CHILDREN UNDER 5 YEARS IN MOZAMBIQUE FOLLOWING DISTRIBUTION OF LONG-LASTING INSECTICIDAL NETS

Erica A. Wetzler

World Vision, Inc., Seattle, WA, United States

Use of long-lasting insecticidal nets (LLINs) is recognized as one of the most effective ways to reduce malaria morbidity and mortality, especially in children, and the universal coverage campaign (UCC) of LLINs is a proven intervention toward this goal. From 1997-2011, use of LLINs saved an estimated 55,757 lives of children under 5 in Mozambique, making LLINs the second most impactful intervention on child mortality. Since investment in the UCC began in 2011, more than 36 million LLINs have been distributed, with an additional 12 million planned in 2020. This paper aims to estimate the impact of the UCC for LLINs from 2012-2019, and project lives saved from 2020-2025 if investment and resources are mobilized to sustain UC. From 2012-2020, the population-based model NetCALC was used to predict provincial household LLIN coverage, inputting data from a 2011 baseline survey and subsequent number of LLINs distributed annually. For 2021-2025, sustained universal coverage was assumed (100%). Annual coverage was entered into the Lives Saved Tool (LiST), a multi-cause mathematical model for estimating mortality. The primary impact measure was estimated lives saved in children 0-59 months. Based on the LiST models, 53,812 child deaths were averted between 2012 to 2019. If currently planned quantities of LLINs are distributed in 2020, and UC is maintained from 2021-2025, an additional 64,312 child deaths could be averted. Sustaining UC of LLINs in Mozambique will require substantial resource mobilization. Without continued investment in sustaining coverage, thousands of avoidable child deaths will occur.

266

FACTORS AFFECTING THE DELIVERY OF COMMUNITY BASED INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY: PERSPECTIVES OF HEALTH WORKERS AND BENEFICIARIES IN NKHATABAY AND NTCHEU DISTRICTS IN MALAWI

John Munthali[1], Jobiba Chinkhumba[2], Alinafe Chibwana[2], Kate Wright[3], Ethel Chilima[1], Shelby Cash[4], Julie Gutman[4]

[1]*Management Sciences for Health, Lilongwe, Malawi,* [2]*Malawi College of Medicine, Blantyre, Malawi,* [3]*Management Sciences for Health, Medford, MA, United States,* [4]*Centers for Disease Control and Prevention, Atlanta, GA, United States*

To prevent malaria in pregnancy in areas of moderate to high transmission, WHO recommends provision of at least three doses of Intermittent Preventive Treatment of malaria in pregnancy with sulfadoxine-pyrimethamine (IPTp) after 13 weeks gestation with doses at least one month apart. The Malawi Ministry of Health (MoH) and partners are exploring acceptability of community-based delivery of IPTp (cIPTp) via Health Surveillance Assistants (HSAs) as a new model of care to improve antenatal care (ANC) attendance and increase coverage of pregnant women with three or more IPTp doses. The following were conducted to assess social determinants influencing cIPTp to strengthen intervention effectiveness: a mid-line qualitative assessment including in-depth interviews with ANC providers, HSA supervisors, HSAs, and women 16-49 years old who delivered in the previous 12 months; focus group discussions with HSAs; and a group discussion with district-level MoH staff. 64 people (42 women; 22 men) participated in the assessment. Most women were more likely to return for ANC and take IPTp if they perceived the provider had a positive attitude. Most health workers and women found cIPTp accessible and acceptable. Many women appreciated that cIPTp reduced transportation costs and time. cIPTp can address cultural barriers to traditional care, including stigma associated with teenage pregnancy and unofficial policies requiring male accompaniment. Regular follow-up by HSAs appears to increase both ANC attendance and IPTp uptake. While no facilities reported stock-outs of SP, stock-outs of pregnancy test kits were identified as a factor limiting early pregnancy detection, delaying initial dosing with IPTp. cIPTp is a promising strategy to increase coverage because the intervention is likely to target and reach pregnant women missed by traditional ANC platforms. Social behavior communication change interventions targeting increased uptake of cIPTp and ANC attendance are needed, along with strengthening existing structures such as community health action groups and integrating cIPTp with community-based management of maternal and neonatal care.

267

INTERMITTENT PREVENTIVE TREATMENT OF MALARIA WITH SULFADOXINE PYRIMETHAMINE AND PROVISION OF INSECTICIDE TREATED NETS IN GEITA, TANZANIA: PROVIDER COMMUNICATION AND OPPORTUNITIES

Courtney Nicole Emerson[1], Ryan Lash[1], Ruth Lemwayi[2], Melkior Assenga[2], Alen Kinyina[2], Annette Almeida[2], Samwel L. Nhiga[3], Lia Florey[4], Chonge Kitojo[5], Erik Reaves[6], Miriam Kombe[5], Peter Winch[7], Stephanie Suhowatsky[7], Mary Drake[2], Julie Gutman[1]

[1]*US Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Jhpiego, Dar es Salaam, United Republic of Tanzania,* [3]*National Malaria Control Program, Tanzania, Dar es Salaam, United Republic of Tanzania,* [4]*US Agency for International Development (USAID), Washington DC, DC, United States,* [5]*US Agency for International Development (USAID), Dar es Salaam, United Republic of Tanzania,* [6]*US Centers for Disease Control and Prevention, Dar es Salaam, United Republic of Tanzania,* [7]*Jhpiego, Baltimore, MD, United States*

Intermittent preventive treatment of malaria in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) is a life-saving intervention to reduce morbidity and mortality among pregnant women and their infants. Additionally, provision and use of insecticide treated nets (ITNs) to prevent malaria is critical to improving pregnancy outcomes. To assess implementation of malaria in pregnancy services and related health communications, we surveyed 1111 women who had delivered a live born infant in the preceding 12 months (recently pregnant women), as well as 1194 adults from randomly selected households without a recently pregnant woman in Geita Region, Tanzania in 2019. Most (88.2%) recently pregnant women reported receiving any IPTp dose; 45.5% received 3 doses. 72.3% of women received their first dose in the second trimester, as recommended by national guidelines, but only 14.4% received IPTp in the 4th month; 20.3% of women did not receive IPTp until third trimester. There was a significant difference between ITN ownership and use among households (HH) with and without a recent pregnancy: ownership of at least one net was 95.2% vs 87.9%, respectively ($p<0.0001$), and use was 90% vs 77.8%, respectively ($p<.0001$). Despite this, few HHs had enough ITNs to cover all residents; on average, HHs had 1 ITN for every 3 rather than every 2 people, as recommended. Notably, only 21.2% and 26.2% of HH with and without a recent pregnancy had sufficient ITNS ($p=0.005$), despite 87.3% of recently pregnant women receiving an ITN during their last pregnancy. Of recently pregnant women, 87% received advice on preventing malaria from a health worker. Of these, 82.7% were advised to sleep under an ITN, but only 66.4% were advised to take SP, and 52.1% to attend ANC regularly. Although uptake of any IPTp was high, there are critical messages that need to be more consistently communicated to pregnant women by ANC providers including the importance of attending ANC regularly during pregnancy. To improve outcomes among pregnant women, additional net distribution may be warranted due to the unexpectedly low access.

268

FACTORS ASSOCIATED WITH ACHIEVING ANTENATAL CARE (ANC) ATTENDANCE AND INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY (IPTP) RECOMMENDATIONS IN GEITA REGION, TANZANIA, 2019

R. Ryan Lash[1], Ruth Lemwayi[2], Melkior Assenga[2], Alen Kinyina[2], Annette Almeida[2], Samwel L. Nhiga[3], Lia Florey[4], Chonge Kitojo[5], Erik Reaves[6], Miriam Kombe[5], Ally Mohamed[3], Japhet Simeo[7], Stephanie Suhowatsky[8], Mary Drake[2], Julie Gutman[1]

[1]*US Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Jhpiego Tanzania, Dar es Salaam, United Republic of Tanzania,* [3]*National Malaria Control Program, Tanzania, Dar es Salaam, United Republic of Tanzania,* [4]*US Agency for International Development, Washington, DC, United States,* [5]*US President's Malaria Initiative / US Agency for International Development Tanzania, Dar es Salaam, United Republic of Tanzania,* [6]*US Centers for Disease Control and Prevention / US President's Malaria Initiative Tanzania, Atlanta, GA, United States,* [7]*Regional Medical Office, Geita, Geita, United Republic of Tanzania,* [8]*Jhpiego, Baltimore, MD, United States*

Malaria in pregnancy results in an estimated 10,000 maternal and 100,000 infant deaths globally each year. To reduce this burden, the World Health Organization (WHO) recommends pregnant women in high to moderate malaria transmission areas receive at least 3 doses of intermittent preventive treatment in pregnancy (IPTp3) with sulfadoxine-pyrimethamine (SP) starting in the second trimester as part of routine antenatal care (ANC). Tanzania has national coverage goals of 80% coverage for women receiving IPTp3 and at least four ANC visits (ANC4). We surveyed women 15-49 years who had given birth in the last 12 months from randomly selected households across 40 communities in Geita Region, Tanzania. ANC attendance and IPTp uptake was recorded from respondent ANC cards if available, or self-reported. Predictors of ANC4 and IPT3 uptake were identified using logistic regression modeling, accounting for clustering and controlling for gravidity. Of 1,111 women surveyed, 505 (51.9%) received IPTp3 and 472 (43.4%) achieved ANC4. Among women who achieved ANC4, 295 (62.5%) received IPTp3. IPTp3 was associated with basic knowledge about ANC and IPTp (aOR 2.4, CI 1.9 -3.1), initiating ANC <20 weeks (aOR 1.7, CI 1.3-2.3), waiting at the facility for <120 minutes (aOR 1.4, CI 1.1-1.9), and receiving advice from a health worker about SP (aOR 1.7, CI 1.3-2.2). ANC4 was associated with better access to care (aOR 1.9, CI 1.3-2.8, for travelling <3.75 km to ANC and aOR 1.9, CI 1.1-2.2, for waiting <90 minutes for the provider), initiating ANC at <20 weeks gestation (aOR 10.7, CI 8.2-14.1), and basic knowledge about ANC and IPTp (aOR 1.4, CI 1.0-1.9). Poor access to care and late initiation of ANC reduced the likelihood that women will attend 4 ANC visits. Knowledge was a predictor of both ANC attendance and IPTp uptake; increasing women's health literacy may overcome some of the barriers associated with retention in ANC. New approaches to delivering ANC that focus on improving knowledge and the experience of care among ANC clients could help close coverage gaps for ANC4 and IPTp3 in Tanzania.

269

INTRODUCTION OF SEASONAL MALARIA CHEMOPREVENTION IN TWO NORTHERN HEALTH ZONES OF BENIN LEADS TO SIGNIFICANT REDUCTIONS IN MALARIA CASES REPORTED IN PUBLIC HEALTH FACILITIES

William Houndjo[1], Hortense Kossou[1], Camille Houetohossou[1], Sakariaou Kpanou[1], Christian Godjo[1], Véronique Adjibi[1], Vivien Akan[1], Augustin Kpemasse[1], Marie-Agnès Agboton[2], Floride Niyuhire[3], Fortuné Dagnon[4], Patrick Condo[4], Bertille Onambele[4], Cyriaque Affoukou[1], Aurore Hounto[1]

[1]*Programme Notional de Lutte contre le Paludisme, Cotonou, Benin,* [2]*ISHA, Cotonou, Benin,* [3]*IHSA, Cotonou, Benin,* [4]*PMI Bénin, Cotonou, Benin*

Malaria has a disproportionate effect on children ages 3 to 59 months in Benin, representing 39.7% of uncomplicated cases reported and 10% of severe cases admitted in public health facilities. While proper management of malaria in health facilities is a priority, care-seeking behavior is weak and many children do not receive care in the recommended time, with the risk of fatal outcome increased. To reduce the malaria burden in children aged 3 to 59 months, Benin began implementation of seasonal malaria chemoprevention (SMC) in 2019, with treatment doses of amodiaquine and sulfadoxine-pyrimethamine (AQ+SP) administered at one-month intervals during peak transmission in eligible zones. Two northern health zones were selected: Malanville-Karimama (MK) and Tanguiéta-Matéri-Cobly (TMC). The SMC campaign was conducted during the months of July, August, September and October 2019 after activities of mapping all eligible children ages 3 to 59 months, training community health workers (CHW) and sensitizing communities and leaders were completed. A total of 116,269 children were identified and administered a three-day course of AQ+SP either directly by CHWs or by caregivers in the presence of CHWs, with between 95.6% and 97.9% of eligible children treated each of the four rounds. The results showed significant reductions (almost 50%) in malaria cases notified in public health facilities in the two SMC zones

comparing the same months in 2018 (pre-SMC) and 2019 (post-SMC). In MK 5,968 cases were notified in 2018 to MK compared to 3,520 cases in 2019 and in TMC 26,992 cases were notified in 2018 in compared to 14,950 in 2019. This indicates that SMC is an effective intervention at reducing malaria cases among children ages 3 to 59 months. Additional analysis of other important outcomes such as mortality in ongoing along with comparison with non -SMC regions. Surveillance needs to continue in the two health zones to monitor epidemiological trends, efficacy of AQ+SP, as well as efforts to improve the quality of care and the collection of quality data.

270

ROLE OF ADVERSE EVENTS ON RELATIONSHIP BETWEEN MALARIA CHEMOPREVENTION DURING PREGNANCY AND NON-ADHERENCE; MEDIATION ANALYSIS OF A RANDOMIZED TRIAL

Noel P. Patson[1], Mavuto Mukaka[2], Titus Divala[3], Lawrence Kazembe[4], Don Mathanga[5], Miriam Laufer[6], Tobias Chirwa[1]

[1]*University of the Witwatersrand, Johannesburg, Johannesburg, South Africa,* [2]*Centre for Tropical Medicine, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom,* [3]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [4]*University of Namibia, Department of Biostatistics, Windhoek, Namibia,* [5]*University of Malawi, College of Medicine, Blantyre, Malawi,* [6]*University of Maryland, School of Medicine, Baltimore, MD, United States*

Treatment non-adherence in clinical trials can lead to selection bias and lower study power. When interventions cause adverse events (AEs), AEs may cause treatment non-adherence or discontinuation. In intermittent preventive treatment during pregnancy (IPTp) trial, antimalarial drug is given to healthy participants such that AE burden can lead to non-adherence (i.e. AE as a mediator). This study investigated the role of AEs on association between IPTp and both treatment non-adherence and participation in IPTp trial. To quantify IPTp indirect effect transmitted via AEs, we conducted mediation analysis using data from 600 pregnant women enrolled in IPTp trial in Malawi and randomly assigned to chloroquine or sulfadoxine pyrimethamine. IPTp non-adherence was defined as missing any dose, after the first dose and study non-completion as discontinuing participation in the trial prior to delivery. AE-intensity, derived as the sum of severity grades of all AEs experienced by a patient between first dose and delivery, proxied overall AE occurrence. Two mediation analyses were done: (i) For time to study non-completion outcome, we fitted accelerated failure time model with IPTp, AE intensity as exposure and mediator respectively; and (ii) for treatment non-adherence outcome, we fitted logistic regression with IPTp and AE occurrence as exposure and mediator respectively. Overall, 474 (79%) participants experienced at least an AE. We documented 81 (14%) treatment non-adherent participants and 146 (24%) who discontinued participation. AE occurrence decreased the likelihood of treatment non-adherence (aOR: 0.24; 95% CI: 0.11, 0.53; p<0.001) and mediated the association between IPTp and treatment non-adherence (p=0.049). Although AE intensity was associated with time-to-study non-completion (β: 0.33; 95% CI: 0.07, 0.58; p=0.012), it did not mediate (p=0.060) the association between IPTp and time to study non-completion. AEs reduced IPTp non-adherence. This may be due to better health care experienced after AEs occurrence in trial setting compared to routine clinical practice such that further work needs to elucidate this interpretation.

271

EFFECT OF SEASONAL MALARIA CHEMOPREVENTION ON MALARIA INDICATORS IN CHILDREN FROM 5 TO 14 YEARS IN DANGASSA MALI EFFECT OF SEASONAL MALARIA CHEMOPREVENTION ON MALARIA INDICATORS IN CHILDREN FROM 5 TO 14 YEARS IN DANGASSA MALI

Drissa Konate, Sory I. Diawara, Bourama Keita, Seydou Doumbia, Mahamadou Diakite

MRTC, Bamako, Mali

The national malaria control program (NMCP) of Mali recommended seasonal malaria chemoprevention (SMC) since 2012 in children under five years. The benefit effects of this strategy on uncomplicated and severe malaria cases has been reported in the target population. However, an increase in malaria incidence was observed in children more than five years in our recently reports. The goal of this study was to assess the change in malaria indicators during the 2019 SMC campaign in children 5-14 years in Dangassa. Since 2017, a new cohort study was conducted in Dangassa village as part of ICEMR project. In collaboration with the NMCP, SMC was implemented in children during this project. A monthly passage was done to collect data in 508 children (253 for intervention arm & 255 for control selected in July) from July to December 2019. The logistic regression model was used to compare malaria risk between the two arms. The monthly coverage rate was 78%, 94%, 84% and 95% respectively in August, September, October & November 2019. In July, the prevalence of *Plasmodium* infection was similar between intervention & control (26.9% vs 31.8, p=0.056). A decrease of 85% (p=0.001) in October (malaria pic) & 82% (p=0.001) in December (one month after stopping SMC) were observed in intervention arm. The risk of anemia was 1.32 (95%CI =0.89-1.99) in July in control arm. An increase of anemia risk was observed in October (OR=2.25 95%CI=1.14-3.57) & December (OR=2.79 95%CI=1.62-4.80) in control arm during the 2019 SMC campaign. The mains side effects reported during the interviews of parent/guardian after drug administration were fever (12.6%), vomiting (10.7%) and drowsiness (7.1%). Almost all parents/guardian had a positive feeling & whish SMC continuation (97.2%). In conclusion, SMC decrease significantly *Plasmodium* infection and anemia in children from 5-14 years living in Dangassa.

272

COMPARATIVE ANALYSIS OF SOME MALARIOLOGICAL PARAMETERS IN SELECTED COMMUNITIES IN THE EAST AND WEST OF BENUE STATE, MIDDLE BELT, NIGERIA: IMPLICATIONS FOR LOCALE SPECIFIC CONTROL INTERVENTIONS

Celina O. Aju-Ameh

-University of Jos, Jos, Nigeria

A key lesson learnt from the failed first global malaria eradication attempt is, one size control strategy no longer fits all. Control interventions need to be tailored to locale specifics. Against this backdrop, this study delved into exploring a dataset from eastern and western communities of Benue State seeking similarities and variations indicative of locale-specific control strategies. Key epidemiological investigations were carried out to determine vectors and parasite species identity, distribution; transmission indices and the prevailing malaria situation. Simultaneously, a cross-sectional survey to elicit information on malaria-related knowledge, attitude and practices was conducted. Molecular assays revealed *Anopheles gambiae, Anopheles coluzzii* and *Anopheles arabiensis* as the dominant malaria vector species. Pooled mean abundance of *Anopheles* species showed no significant difference (t = 1.0487). Transmission indices showed no significant difference between means for: Sporozoite Rate (t= -0.91987); Human Biting Rate (t = 0.51508); Entomological Inoculation Rate (t = -1). A higher percentage (57.1%) from Gboko (East) took a first action within the first 24h of onset of symptoms to combat malaria as against (45.5%) from Otukpo (West). Otukpo had no archival malaria ward admission record. Amongst the various source of malaria medication,

patent medicine store peaked (82%) for Gboko and medical doctors (76%) for Otukpo. Same control strategies could be deployed in both communities for vector species and transmission indices. Although Gboko had a higher percentage response within the first 24h; enlightenment programs to advise both communities on the cruciality of early detection and treatment of malaria for improved response is suggested. Deliberate effort is needed to address the absence of historical malaria data in western communities. Behavioral determinants in populations that are detrimental to the uptake of control interventions should be integrated into social communication and addressed per specifics.

273

RESPONSE TO AN INCREASE IN MALARIA TRANSMISSION IN URBAN DISTRICT OF ZANZIBAR

Faiza B. Abbas[1], Naomi A. Serbantez[2], Shabbir M. Lalji[3], Abdulla S. Ali[1], Mohamed Y. Ndee[1]

[1]*Zanzibar Malaria Elimination Program, Zanzibar, United Republic of Tanzania,* [2]*US President's Malaria Initiative, Dar es salaam, United Republic of Tanzania,* [3]*RTI International, Dar es Salaam, United Republic of Tanzania*

Malaria prevalence in Zanzibar is 0.2% with focalized transmission in some areas. Between 2014-2018, the average annual number of malaria cases in Urban district was 712 and incidence was 2.9/1000 population. In 2019, Urban experienced a three-fold increase in malaria cases (1,872) and incidence (8.9/1000). Over 50% of cases were reported between October-December 2019. This period was also wetter with a cumulative precipitation of 8,488 mm compared to 3,261 mm in 2018. 2/57 shehias (Kikwajuni Juu and Bondeni), had the highest incidence of 46 and 49 per 1,000, respectively. The Zanzibar Malaria Elimination Program in collaboration with Council Health Management Teams (CHMTs) and partners, conducted a joint outbreak investigation and response in absence of an Epidemic Preparedness and Response Plan. Review of routine case-based malaria surveillance data was done to assess trends, reported long-lasting insecticide net (LLIN) use, and time from onset of symptoms to diagnosis (health seeking behavior). Mass screening and treatment (MSaT), and larval source management as recommended by World Health Organization (WHO) were undertaken. Sensitization through community meetings and media was done to promote LLIN use and seek early treatment. Surveillance data showed only 15% of the population in the 2 shehias used an LLIN (compared to 50% in Urban district), and only 35% of malaria positive cases sought treatment within 24 hours of onset of symptoms. Results of response activities included: 1) MSaT where 3,785 people were tested for malaria, of whom 103 (2.7%) were positive; 2) distribution of 2,400 LLIN coupons to 968 households; 3) biolarviciding of 3 vector breeding sites; and 4) reach >90% of the population through 5 community meetings, and 15 radio and 12 television spots. By February-March 2020, only 61 cases were reported compared to 450 in December-January period. Response, alongside increased community awareness on prevention and treatment measures may have helped in malaria reduction. This could serve as a basis to develop response guidelines for CHMTs. Community-based sensitization will also mitigate future malaria risk in Zanzibar.

274

ENDING *PLASMODIUM FALCIPARUM* TRANSMISSION IN SURINAME WITH ADAPTIVE AND INNOVATIVE STRATEGIES

Helene Hiwat[1], Hedley Cairo[1], Loretta Hardjopawiro[1], Dayanand Panchoe[2], Malti Adhin[3], Edward Van Eer[4], Marthelise Eersel[3], Oscar Mesones[5], Stephen Vreden[6]

[1]*Malaria Program Suriname, Paramaribo, Suriname,* [2]*Bureau of Public Health Suriname, Paramaribo, Suriname,* [3]*Anton de Kom University Suriname, Paramaribo, Suriname,* [4]*Primary Health Care Suriname, Paramaribo, Suriname,* [5]*PAHO Suriname, Paramaribo, Suriname,* [6]*Academic Hospital Paramaribo, Paramaribo, Suriname*

Before 2006 Suriname had the highest concentration of *Plasmodium falciparum* malaria in the Americas. Various targeted malaria interventions were implemented and adapted over time to the changing national transmission dynamics. This resulted in zero indigenous *P. falciparum* cases reported in the country in 2019. Primary risk populations before 2006 were tribal villagers in the interior of Suriname. The proportion of *P. falciparum* among malaria cases was high (79.7% in 2004). Interventions between 2004 and 2009 (introduction of artemisinin-combination therapy, Active Case Detection surveys (ACDs), Indoor Residual Spraying, health education, free distribution of insecticide-treated mosquito nets (ITNs), resulted in a 67.8% reduction in malaria cases, 80.5% reduction in *P. falciparum* cases and near elimination of malaria in the villages. After 2006, malaria was mainly prevalent among gold miners, a significant proportion of whom where Brazilian. Many traveled back and forth between Suriname and French Guianese mining areas, where malaria risk remained high. Targeted interventions towards those miners included the introduction of a low-threshold migrant clinic with multilingual personnel in the capital; establishing a Malaria Service Deliverers (MSDs) network in the mining areas, with MSDs being recruited from the at-risk populations; ACDs and ITN distribution in remote mining sites; and focused health education taking into account languages and education level of the risk populations. Imported malaria was specifically targeted with malaria screening points at key localities along the French border, and the provision of self-diagnosis and self-treatment kits, together with ITNs, to cross-border moving populations as part of an innovative tri-national pilot study (MALAKIT). Relations with neighboring countries in terms of data sharing and collaborative interventions were strengthened. Sustaining interrupted transmission of *P. falciparum* in Suriname requires a continued investment in the national surveillance and response system to prevent re-introduction and the establishment of a regional malaria elimination goal.

275

TACKLING RE-INTRODUCTION OF *PLASMODIUM VIVAX* MALARIA IN PRE-ELIMINATION SURINAME; A COLLABORATIVE AND INTEGRATIVE PACKAGE OF INTERVENTIONS

Stephen Gerold Vreden[1], Gaitree Baldewsingh[2], Hedley Cairo[3], Irma Jaglall[2], Dayanand Panchoe[4], Malti Adhin[5], Herman Jintie[2], Edward Van Eer[6], Marthelise Eersel[5], Oscar Mesones[7], Helene Hiwat[8]

[1]*Academic Hospital Paramaribo, Paramaribo, Suriname,* [2]*Medical Mission Primary Health Care Suriname, Paramaribo, Suriname,* [3]*Malaria Program Suriname, Paramaribo, Suriname,* [4]*Bureau of Public Health Suriname, Paramaribo, Suriname,* [5]*National Malaria Elimination Task Force Suriname, Paramaribo, Suriname,* [6]*Malaria elimination Task Force Suriname, Paramaribo, Suriname,* [7]*PAHO Suriname, Paramaribo, Suriname,* [8]*Ministry of health Malaria Program Suriname, Paramaribo, Suriname*

Suriname is aspiring malaria elimination; there were only 30 autochtonous infections (25 *Plasmodium vivax* (P.v.), 4 *P. falciparum (P.f.)* one mixed P.f. and P.v.) in 2018. Due to the current regional context, Suriname remains vulnerable for re-introduction of *P. vivax* malaria, which unfortunately happened in March 2019 when malaria was re-introduced in an Indigenous village where malaria had been eliminated for 3 years. Six (6) cases were detected among the population of 420 persons in a period of 6 weeks. The index case was believed to be a visitor from bordering Brazil. The village generally has a high density of *Anopheles darlingi* mosquitos. The outbreak response was a package of collaborative integrated interventions including health education, distribution of impregnated nets, bi-weekly active case detection surveys (ACD), and mass drug administration (MDA). The first MDA consisted of weekly Chloroquine (C) prophylactic treatment for all villagers with a negative blood smear from May through August 2019. All visitors to the village were tested upon arrival and treated accordingly if infected. The aim was to prevent transmission of parasites from humans to mosquitos vice versa. All cases were treated by Direct-Observed Treatment (DOT) with chloroquine and primaquine (P; 0.50 mg/kg, for 14 days). Six weeks after finalization of the MDA, new cases emerged; from September 2019 until January 11, 2020, 43 new cases were detected. In the first week of January 2020 Indoor Residual Spraying was carried out in the village. After January 11 no new

cases were detected. Taking into account the possibility of failure of PQ to prevent relapses, all cases in the village who had been diagnosed with and treated for *P. vivax* malaria in the past months received a full retreatment with (C + P). So far, no new cases were diagnosed. The process of institutional collaboration and multifaceted integrated actions shows to be key in tackling the re-introduction of *P. vivax* malaria in a (pre-) elimination setting.

278

PLASMODIUM FALCIPARUM INFECTION DYNAMICS IN SUBCLINICAL POPULATION

Ruth Ayanful-Torgby[1], Esther Sarpong[2], Dickson Donu[1], Evans Obboh[2], Joshua Adjah[1], Linda E. Amoah[1]

[1]*University of Ghana, Noguchi Memorial Institute for Medical Research, ACCRA, Ghana,* [2]*School of Medical Sciences, University of Cape Coast, Cape Coast, Ghana*

Subclinical *Plasmodium falciparum* infections in endemic populations pose a challenge to current malaria control strategies. Understanding the parasite transmission dynamics in a population could impact the design and implementation of preventive tools. Infection prevalence, sexual-stage antibody levels and diversity were actively investigated in a cohort of school-going children aged 12 years and below at six bimonthly time points (visit) to monitor infection dynamics in an off-peak transmission season. High microscopic parasite prevalence (>28%) but relatively low parasite densities (2324 ± 516 µl), submicroscopic infections (>55%) and gametocytes carriage (>45%) were observed. Submicroscopic parasites and gametocytes persisted in 18% and 3% of the participants respectively throughout the study period. We identified 13 different gametocyte clones by genotyping (mRNA) *Pfg377* locus with low MOI (<1.5), without the same clone in an individual with persisted infections. No differences were noted in IgG and IgM of Pfs4845 and Pfs230 antibody levels in individuals either with persistent, sporadic or no infection, as well as parasite densities/prevalence and gametocyte carriage among the visits. A tenth of the children was never infected, nonetheless, more than 50% of the children had ≥3 infection episodes. The observed changes in sexual clones in individuals with persisted infections could result in gametocyte with different longevity serving as a reproductive strategy to ensure the parasite survival and availability to the mosquito in the off-peak season when the biting rate is low. Hence, a pragmatic approach is needed to defined infections in high-burden individuals within a locality to target and prioritize interventional resources.

279

UPDATES ON MALARIA EPIDEMIOLOGY AND PROFILE IN CABO VERDE FROM 2010 TO 2019: THE GOAL OF ELIMINATION

Adilson De Pina

Ministry of Health, Praia, Cape Verde

Cabo Verde is one of the E-2020 Initiative of 21 malaria-eliminating countries. Located in West Africa, is an archipelagic country with a history of malaria since the settlement of the islands during the 16th century, and with a low incidence in recent years. A secondary data review of all diagnosis cases and reported cases notified to the Ministry of Health and Social Security between 2010 and 2019. Excel sheets were used to was analysed to assess the origin of the malaria cases, analysis by age, sexes and municipality was done. The Incidence, Mortality and Lethality rate was calculated according to the WHO formulas. Analysis of the origin of the cases was made, classifying as indigenous or imported, according to WHO recommendations. A total of 819 malaria cases were reported in the country, being 554 (67.6%) local cases and 263 (32.1%) imported cases, with 02 (0.20%) introduced cases reported in 2018 and 2019, one each. More than half of cases (446/54.5%) were reported during the outbreak in 2017. The majority of cases (766; 93.5%) are malaria simples, affecting especially man (600 cases; 73.3%) with 20 years old and more (658 cases; 79.7%). Cases are reported from the majority of municipalities in the country (14/22; 64%), being the local cases from six municipalities and in the last 3 years, exclusively to the capital, Praia. Imported case are from different countries, specially Lusophony countries (Guinee Bissau and Angola; 24.33% and 22.43% respectively) and other sub-Saharan countries, and in the last years with cases from America (Brazil) and Asia (Philippines). Incidence, mortality and fatality rates had been low, in comparison with the other countries, being the highest value of 0.83, 0.60 and 8.3, respectively. The geographic location of the country, the mobility to and from endemic areas of its populations and migrants, makes the risk of malaria a constant reality. Studies must be continued to follow up the malaria data in the country, allowing better decision-making in the scope of elimination in 2020 horizons.

280

SPATIAL CLUSTERING OF MALARIA RAPID DIAGNOSTIC TEST POSITIVE INFECTIONS AND SUBPATENT PARASITEMIA IN WESTERN KENYA, 2013: IMPLICATIONS FOR FOCAL MASS DRUG ADMINISTRATION IN A SETTING OF HIGH TRANSMISSION

Monica P. Shah[1], Michael R. Kramer[2], Lance A. Waller[3], Wycliffe Odongo[4], Norbert Odero[5], Kephas Otieno[6], Tony Sang[6], Ya Ping Shi[7], Kim Lindblade[7], Aaron M. Samuels[7], Simon Kariuki[6], Meghna Desai[7]

[1]*Malaria Branch, Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention; Department of Epidemiology, 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,* [3]*Department of Biostatistics and Bioinformatics, Rollins School of Public Health, Emory University, Atlanta, GA, United States,* [4]*Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya; Malaria Branch, Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, GA, United States,* [5]*Duke Global Health Institute, Durham, NC, United States,* [6]*Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya,* [7]*Malaria Branch, Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, GA, United States*

Focal mass drug administration (fMDA) leverages the spatial heterogeneity of malaria transmission to more efficiently target individuals at greatest risk of infection. An index case is identified by a positive rapid diagnostic test (RDT) during passive surveillance and both the individual and household members are treated with antimalarials. FMDA effectiveness may depend on the spatial clustering of index case and subpatent infections, detected by polymerase chain reaction (PCR). The present study aimed to characterize compound-level (social unit of multiple households) clustering of malaria infections to describe the potential impact of fMDA strategies in a high transmission area of western Kenya. During the first round of a mass test and treat trial, >23,000 individuals were tested by RDT; among RDT negatives, 4785 samples were randomly selected for PCR testing. The analytic dataset included only sampled participants and their compound members (n=2,572 compounds, n=16,386 individuals). Log binomial regression was used to explore the association between either at least one RDT positive or febrile compound member (based on previous 48-hour self-report, irrespective of RDT result) with subpatent infection in the compound, controlling for age (a resident under five years of age) and enhanced vegetation index. Among sampled participants, 13.5% were PCR positive. Among compounds with a sampled participant, 74% had at least one RDT positive case, 49% had at least one febrile member, and 23% had at least one subpatent infection. Compounds with a RDT positive case were significantly more likely to have a subpatent infection than compounds with all RDT negatives (odds ratio [OR] 1.5, 95% confidence interval [CI]: 1.2-1.8, p<0.001). Similarly, compounds with a member reporting recent fever were significantly more likely to have subpatent infection (OR 1.4, 95% CI 1.2-1.6, p<0.001). Although there was an association between compound-level RDT positive or febrile

infections with subpatent infections, the high proportion of RDT positive case compounds could limit the utility of a potential approach such as fMDA in a high transmission setting.

281

MALARIA TREATMENT ADHERENCE AMONG GOLD MINERS IN GUYANA: DO BELIEFS MATTER?

Bolanle B. Olapeju[1], Camille Adams[1], Gabrielle Hunter[1], Sean Wilson[1], Joann Simpson[1], Lyndsey Mitchum[1], TrishAnn Davis[1], Horace Cox[2], Helen Imhoff[2], Neil Trotman[2], Jen Orkis[1], Doug Storey[1]

[1]*Johns Hopkins Center for Communication Programs, Baltimore, MD, United States,* [2]*Guyana National Malaria Program, Georgetown, Guyana*

The Guyana National Malaria Program (NMP) is rolling out its community case management initiative, using trained malaria testing and treatment volunteers to curb malaria transmission among remote gold mining populations within Regions 1, 7 8 and, 9. Miners in these regions have historically resorted to self-diagnosis and non-traditional treatment. The USAID-funded Breakthrough ACTION project conducted a baseline survey among 1685 miners from 233 mining camps in Regions 1,7 and 8 prior to scaling up social and behavior change (SBC) interventions among the trained volunteers. About 30% of all miners reported at least one malaria episode in the past year. Multivariate logistic regressions were used to explore correlates of self-reported malaria treatment adherence in the past year accounting for their sociodemographic characteristics, malaria-related knowledge, beliefs, and SBC message exposure. Malaria treatment adherence was highly associated with miners' belief in their ability to complete prescribed antimalarial treatments (aOR: 2.91; 95% CI: 1.90, 4.45) and beliefs regarding their friends/coworkers' treatment adherence (aOR: 2.12; 95% CI: 1.20, 3.74). No other covariates explored were significantly associated with treatment adherence. The findings suggest that while the expansion of malaria services may make testing and treatment more accessible to miners, additional social and behavior change efforts may be needed to improve miners' beliefs towards malaria treatment and address their perceived barriers to adherence. To highlight the importance of treatment adherence, the Breakthrough ACTION project, with the support of NMP is rolling out user-friendly treatment adherence handouts to miners with malaria that demonstrate how the parasites reduce as malaria treatment completed.

282

TARGETED RESPONSE TO MALARIA IN THE HIGHEST BURDEN PROVINCES OF CAMBODIA: ONGOING SUCCESS OF THE INTENSIFICATION PLAN

Siv Sovannaroth[1], Pengby Ngor[1], Vichka Khy[2], Julia C. Dunn[3], Michelle Pahl[2], Huy Rekol[1]

[1]*Cambodia National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia,* [2]*CHAI, Phnom Penh, Cambodia,* [3]*CHAI, Vientiane, Lao People's Democratic Republic*

Since Oct 2018, the Cambodia National Center for Parasitology, Entomology and Malaria Control (CNM) and partners have implemented an Intensification Plan (IP), targeting a suite of interventions to areas with the highest burden of all species of malaria to interrupt transmission and reverse the increase in cases seen in 2018 (125% increase Jan-Jul 2018 compared to 2017). Over the first phase (IP1, Oct 2018-Sep 2019) there was a 72% decrease in *Plasmodium falciparum* (Pf) cases and 122% increase in testing. Interventions in IP include mobile malaria workers (MMWs) providing 24-hour access to diagnosis and treatment services and single low dose primaquine, behaviour change activities, and enhanced supply chain. The IP also targets high risk forest-goers with active case detection and provision of forest-packs with long-lasting insecticide treated hammock nets (LLIHN). The Intensification Plan 2 (IP2, Oct 2019 - Dec 2020) builds on the success of IP1 by targeting the health centres (HCs) with the highest number of Pf cases (69% of all Pf cases in country) and adding the provision of mosquito repellent and village malaria worker (VMW) outreach to villages. Central CNM staff analyse the malaria data on a monthly basis and staff at provincial, district and HC levels directly supervise activities. There have been 4985 malaria cases during IP2 so far (Oct 2019 - Feb 2020), a 50% decrease from the same period the previous year; a 66% decrease in Pf/mix cases and a 44% decrease in *P. vivax* cases. During IP2, testing has increased by 10% and was mostly attributable to MMWs (54% increase) and VMWs (22% increase). In Feb 2020, 17 HCs reported zero Pf/mix cases compared to 4 HCs in Oct 2019. Additionally, 28,486 LLINs have been distributed to households, and 4650 forest packs have been distributed to forest-goers. The programmatic gains of IP2, including more dedicated malaria staff and increased testing, have seen a decline in malaria cases, continuing from IP1. Central and provincial monitoring of IP2 will continue to ensure that gains are maintained into the rainy season, when risk of infection is higher, and to supervise effective implementation of interventions.

284

EXPANDING MULTI-SECTORAL PROGRAMMING FOR MALARIA ELIMINATION: INITIAL LESSONS FROM MALARIA AND NUTRITION COMBINED PROGRAMMING IN MADAGASCAR

Joseph Lewinski[1], James Hazen[2], Suzanne Van Hulle[3], Tanja Englberger[2], Benjamin Rice[4], Carla Fajardo[2], Christopher Golden[4]

[1]*Catholic Relief Services, Baltimore, MD, United States,* [2]*Catholic Relief Services Madagascar, Antananarivo, Madagascar,* [3]*Catholic Relief Services, Baltimore, DC, United States,* [4]*Harvard University, Cambridge, MA, United States*

Multi-Sectoral programming is the foundation for WHO's High Burden to High Impact (HBHI) approach, which coincides with Catholic Relief Services (CRS) support to low-income countries in health, emergencies, agriculture, water and environment, education, and governance. Building on over 16 years of malaria programming experience and several decades a multisectoral agency, CRS is investing in expanding the knowledge and use of multi-sectoral malaria activities in line with the HBHI approach. Phase 1 of this strategic investment has focused on expanding the role of malaria interventions within nutrition programs in Madagascar. Several direct and indirect pathways link malaria and nutrition outcomes. Ongoing research in Mananjary District, in Madagascar has shown that areas with high malaria prevalence overlap with areas with poor nutritional status. Across surveyed communities in southeastern Madagascar, 50.98% to 92.16% of households were observed to have at least one member of the household infected with malaria. At the individual level, 3.64 to 46.18% of individuals across all ages tested positive for malaria. This is in contrast to regional averages that were substantially lower, 3 to 9% for children, in the 2016 MIS. From the preliminary data analysis, 27.56 to 68.12% of individuals across these study communities were found to be anemic and 57.1% of children were stunted. Households commonly reported having to reduce the quality of diet due to insufficiency, both by restricting consumption by children and by reducing food quality. The same research conducted in southwest Madagascar showed high rates of malaria, moderate rates of acute malnutrition and lower rates of stunting which provides further insight on how malaria and nutrition co-infection play out in different ecological zones with different diets, norms and weather patterns. From these results, integrated food security programming was conducted in Mananjary to improve dietary diversity, malaria prevention and treatment and expand hygiene and sanitation services to help reduce the high rates of stunting.

285

PILOTING AND EVALUATING AN ELECTRONIC TOOL FOR MALARIA SUPPORTIVE SUPERVISION IN MOZAMBIQUE

Baltazar N. Candrinho[1], Ana Cristina Castel-Branco[2], Ana R. Chico[2], Nicole Dagata[3], Mércia Dimene[1], Eliza Walwyn-Jones[4], Samira Sibindy[1], Mariana da Silva[5], Rose Zulliger[6]

[1]*National Malaria Control Program, Maputo, Mozambique,* [2]*Clinton Health Access Initiative, Maputo, Mozambique,* [3]*Clinton Health Access Initiative, Mbabane, Swaziland,* [4]*Clinton Health Access Initiative, Gabarone, Botswana,* [5]*Malaria Consortium, Maputo, Mozambique,* [6]*President's Malaria Initiative, Centers for Disease Control and Prevention, Maputo, Mozambique*

Malaria remains a public health challenge in Mozambique. In the 2018 Health Facility Survey, only 29% of suspected malaria cases were correctly managed. Similarly, National Malaria Surveillance assessments from 2017-2018 revealed lack of data integration and standardized reporting leading to poor data use. To address these operational gaps, the National Malaria Control Program (NMCP) developed a system aimed at strengthening malaria program implementation through routine central to province, province to district and district to facility/community mentorship by NMCP supervisors. Electronic checklists with case management, vector control and data quality assessment content guide the supervisors through mentorship and systematic collection of data. A 4-day pilot of the program tested the supervision checklists in five health facilities (HF) in Manica and Maputo Provinces. HF were selected to include urban and rural areas and high and low patient volume. A total of 11 supervisors responded to a questionnaire included in the tool for feedback on content and usability. Responses were collected and analyzed using Kobo Toolbox. The questionnaire received 23 responses as some supervisors managed multiple checklists. The main findings from the pilot focused on improving the checklists flow by consolidating and/or reorganizing questions. Feedback was also given on technical content. 52.2% of questionnaire responses found the technical prompts helpful to guide mentorship. Overall findings highlighted the need to track progress over time by adding scores to the tool's next version. The NMCP aims to enhance health outcomes through mentorship and evidence-based interventions. The electronic tool supports this through feedback prompts and action-planning components that provide guidance to health workers and ongoing assessment of HF issues. This experience of supervision tool development provides an example for how national programs might promote mentorship and data use to improve malaria programming and impact. Next steps are national scale-up of the tool with routine assessments to ensure it is addressing gaps in the health system.

287

SPATIAL ANALYSIS OF MALARIA SEROPOSITIVITY IN AN AREA OF RAPIDLY DECLINING TRANSMISSION, NORTHERN LAO PDR (LAOS)

Estee Y. Cramer[1], Emily Dantzer[2], Francois Rerolle[2], Lindsey Wu[3], Bouasy Hongvanthong[4], Adam Bennet[2], Andrew Lover[1]

[1]*UMass Amherst, Amherst, MA, United States,* [2]*UCSF Global Health Group's Malaria Elimination Initiative, San Francisco, CA, United States,* [3]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [4]*Center of Malariology, Parasitology, and Entomology, Vientiane, Lao People's Democratic Republic*

As a result of elimination initiatives, malaria incidence in Lao PDR has decreased 80% from 2010- 2017. Approximately half of the reported cases are *Plasmodium. vivax* and half *P. falciparum*. As the parasite burden decreases, many malaria cases are asymptomatic and thus are not reported to health centers. In settings like this, serology can be a useful complement to parasite-based metrics to identify transmission hotspots. Using data from a 2016 cross sectional survey in four districts across Northern Laos, a multiplex immunoassay was used to detect antibody levels against PvAMA1 (a marker of malaria exposures) in 4,981 participants. Cut-off values were used to categorize samples as seropositive or seronegative for this antibody. A log-transformed linear regression model was used to describe the relationship between an individual's age and antibody response. Descriptive tests and geographically weighted models were used to assess clusters of participants (hotspots) where antibody response (indicative of *P. vivax* transmission) was higher than anticipated. Based on a linear model, age accounts for 13% of the variation in expected serology response to PvAMA1. After adjusting for occupation, 22.5% of the variation can be accounted for in an aspatial linear model. Moran's I indicates that there is significant spatial autocorrelation of antibody responses ($p < .0001$) and the residual values ($p < .0001$). A Bernoulli model of the binary outcome (SaTScan), identified 15 clusters of a higher than expected number of seropositives, the majority of which are in Paktha district (Bokeo province) and along the Mekong river. Identifying hotspots is beneficial for targeted malaria monitoring because recent transmission may have occurred there. Additionally, Paktha borders Thailand so these hotspots may be used to track transmission across international borders. In order to reach elimination goals by 2030, novel tools are needed to detect residual transmission. By analyzing clusters of higher than expected antibody responses, locations of continued transmission can be identified and targeted for intensive interventions.

288

IMPLEMENTING PRIMAQUINE RADICAL CURE IN CAMBODIA: EVALUATING IMPACT ON *PLASMODIUM VIVAX* INFECTION AND RELAPSE, IDENTIFYING OPERATIONAL ROADBLOCKS AND MAXIMIZING EFFECTIVENESS

Dysoley Lek[1], Yucheng Tsai[2], Jillian Dunning[2], Siv Sovannaroth[1], Or Vanthen[3], Srey Sin[4], Kros Sarath[5], Prak Vonn[6], Keo Sophaktra[7], Top Sophorn Narann[8], Nguon Sokomar[8], Huy Rekol[1], Evelyn Wong[2], Michelle Pahl[2]

[1]*National Center for Parasitology, Entomology and Malaria Control, Phnom Pehn, Cambodia,* [2]*Clinton Health Access Initiative, Phnom Pehn, Cambodia,* [3]*Provincial Health Department, Ministry of Health, Kampong Speu, Cambodia,* [4]*Provincial Health Department, Ministry of Health, Kampong Thom, Cambodia,* [5]*Provincial Health Department, Ministry of Health, Siem Reap, Cambodia,* [6]*Provincial Health Department, Ministry of Health, Kampong Chhnang, Cambodia,* [7]*Provincial Health Department, Ministry of Health, Banteay Meanchey, Cambodia,* [8]*Cambodia Elimination Malaria Project, Phnom Pehn, Cambodia*

The number of malaria cases in Cambodia has decreased by 64% from 2010 to 2019. Whilst reported cases of *Plasmodium vivax* (Pv) have decreased by 23% in that time period, Pv cases accounted for 84% of malaria cases in 2019. Patients with Pv infection may suffer relapse due to dormant liver stages. To prevent this, glucose-6-phosphate dehydrogenase (G6PD) testing and 14-day primaquine radical cure has been introduced to treat G6PD-normal male patients. The first phase of rollout will take place in 88 health facilities in four provinces from October 2019 to September 2020. We will analyze operational and epidemiological indicators to improve radical cure provision and to determine the impact of radical cure on interrupting transmission. To further evaluate whether radical cure impacts Pv relapse, a set timeframe will be applied to retrospective patient data from January 2018 onwards to identify infections as primary, reinfection or relapse. From October 2019 to February 2020, 463 male Pv and mixed species patients received G6PD tests. Of these, 346 (75%) were G6PD-normal and all received radical cure treatment with 92% patients adherent to the full course of treatment. During this time, rollout provinces have seen a reduction of 241 Pv cases, compared to a decrease of 63 cases in the same time period across control provinces with similar malaria incidence. As data collection continues this will be updated to ensure that reductions are maintained through the rainy season and the results of analysis of relapse cases will be incorporated. The first phase of rollout has yielded important operational lessons for the national program, for example issues of losses in referral from community to health center, variable G6PD testing, concerns with G6PD interpretation, and patient refusals. Five adverse events were recorded, however investigation determined that none were caused by radical cure. Improving availability of radical cure in Cambodia should reduce Pv relapse and reduce onward

transmission. The lessons learnt in the first phase of rollout will inform the national scale up of radical cure to support efforts to eliminate malaria by 2025.

289

STRENGTHENING THE SENSITIVITY OF SURVEILLANCE SYSTEM FOR MALARIA ELIMINATION IN MUNAUNG TOWNSHIP, RAKHINE STATE OF MYANMAR

Khin T. Win[1], Hein H. Nyan[2], Aung Thi[3], Thant Z. Aung[2], Sai H. Pha[2], Saw Lwin[1], Phyoe Yarzar[1], May A. Lin[1], Neeraj Kak[4], Ye Hein Naing[1]

[1]*University Research Co., LLC, Yangon, Myanmar,* [2]*University Research Co., LLC, Toungup, Myanmar,* [3]*University Research Co., LLC, Nay Pyi Taw, Myanmar,* [4]*University Research Co., LLC, Chevy Chase, MD, United States*

The President's Malaria Initiative funded Defeat Malaria Project to conduct the collaborative pilot malaria elimination interventions in Munaung Township of Rakhine State was approved by Core Technical Strategic Group and Ministry of Health and Sports. The activities were initiated in 2018 in coordination with of Basic Health Staff (BHS) to meet the national milestone of eliminating *Plasmodium falciparum* species by 2025. Munaung is the second biggest island in Rakhine State located at Bay of Bengal with an area of approximately 523 km^2 and composing of 139 wards/villages. Due to the drastic reduction in malaria cases in Munaung (7 cases in 2015, 3 cases in 2016 and 0 case in 2017), Annual Blood Examination Rate (ABER) decreased from 8.7% in 2015, 2.4% in 2016, and 1.8% in 2017. The Township Health Department in collaboration with Defeat Malaria intended to increase the sensitivity of surveillance system by targeting ABER at 10% to prove the malaria free situation despite of adequate testing for those with suspected symptoms of malaria. It is also vital to be maintained through prevention of reintroduction of malaria by mobility monitoring and timely response. In addition, 99 villages with no fixed health facilities were covered by 60 BHS for active case detection through weekly fever surveillance and prevention of onward transmission if an index case is identified. Defeat Malaria assisted the local health department in collecting and analyzing the reports on a monthly basis. Regular joint supervision visits and beneficiaries' interviews were conducted for on-site data verification. On a monthly basis, Defeat Malaria supported the surveillance information to the Township Medical Officer for appropriate actions. As a result, the proportion of villages with ABER ≥10% was dramatically increased from 3% in 2017 to 94% in 2018 and 89% in 2019 resulting in the total ABER of Munaung Township improving from 1.8% in 2017, to 15.5% in 2018, and 17.7% in 2019. In spite of this increased surveillance, no malaria cases were discovered in 2018 and only one imported case was detected in 2019.

290

PILOTING AND EVALUATING A SUITE OF DIGITAL SOLUTIONS FOR MALARIA ELIMINATION

Anne Liu[1], Vivek Agrawal[1], Pedro Pagalday Olivares[1], Sameen Babur[1], William Avilés[1], Juan Manuel Alcantara Acosta[2], Jose García Muñoz[2], Karoline Tufte Lien[2], Marta Vila[2], Rodolfo Melia[2], Derek Treatman[3], Pierre Dane[3], Anna Winters[4], Anne Martin[4], Matt Berg[5], Craig Appl[5], Mwalenga Nghipumbwa[6], Abdisalan Noor[6], Arnaud Le Menach[1], Lakshmi Balanchandran[1]

[1]*Clinton Health Access Initiative, Boston, MA, United States,* [2]*University of Oslo, Oslo, Norway,* [3]*Vital Wave, Palo Alto, CA, United States,* [4]*Akros, Lusaka, Zambia,* [5]*Ona, Burlington, VT, United States,* [6]*World Health Organization, Geneva, Switzerland*

In 2018, a consortium of partners developed a suite of digital tools to strengthen malaria surveillance systems in response to gaps identified by the World Health Organization (WHO) and National Malaria Programmes across the Greater Mekong Sub-region, Sub-Saharan Africa, and Mesoamerica. Tools were developed in 2018-2019 to improve user-friendliness, data timeliness, availability of geospatial data, task management, and integration of system. This suite of tools includes enhancements to the DHIS2 web platform (the malaria information system in many countries), DHIS2 Android Capture Application (to support case notification and investigation, and focus investigation), and Reveal (to support focus investigation and intervention responses). In 2019-2020 components of the suite were piloted in Honduras, Thailand, Namibia, and South Africa to support case notification, case investigation, focus investigation, indoor residual spraying, and analytics respectively. A monitoring and evaluation (M&E) framework developed with the WHO Global Malaria Programme informed methods to assess performance and impact of the tools on malaria surveillance in 2020. Database analysis was conducted comparing the new digital tool with legacy systems. Furthermore, qualitative interviews were planned with end users and key government stakeholders. Indicators were collected on system performance, hardware performance, user profile and usage, user engagement and usability, data quality and reporting, surveillance process impact, governance, and sustainability. Preliminary results in the pilot countries have demonstrated increases in user engagement and the use of data to drive decision making, improvements in data timeliness and investigation rates, and higher data quality compared to legacy systems. Results also pointed to improvements needed in supervision, training, and user friendliness of the tools to address syncing issues and foci investigation rates. M&E is expected to be completed and results compiled by the end of 2020, and results will inform further strengthening of the tools to support malaria surveillance systems.

291

IMPROVING THE QUALITY AND PRIORITIZATION OF MALARIA CASE MANAGEMENT RESOURCES THROUGH THE IMPLEMENTATION OF A DIGITAL SUPERVISION TOOL IN THE PUBLIC HEALTH SECTOR OF 6 PROVINCES OF ANGOLA, 2019-2020.

Jose Franco Martins[1], Rukaaka Mugizi[2], Christopher Lourenco[3], Paulo Proto[2], Luis Bolanos[2], Anya Fedorova[2], Victor Lara[3], Claudia Ramos[2], Admilde Jose[2], **Benjamin Nieto-Andrade**[2]

[1]*National Malaria Control Program / Angola, Luanda, Angola,* [2]*PMI Angola - Health for All Project, Population Services International / Angola, Luanda, Angola,* [3]*Population Services International / Washington, Washington, DC, United States*

Malaria accounts for 35 percent of curative care demand in Angola, and causes 60 percent of hospital admissions in children less than five years of age. The Angolan National Health Development Plan 2012-2025 recognizes the difficulties in ensuring quality health services due to the lack of a systematic process for evaluating health units and prioritizing capacity building for health providers. The Ministry of Health (MoH) in collaboration with Health For All piloted a digital supervision tool for malaria in health facilities in six provinces with the highest malaria burden in Angola in 2019. The tool used checklists aligned to Angolan medical standards and included quality of care questions to assess malaria case management (MCM), commodity availability, and facility data management. Seven workshops were conducted between April and September 2019 to train 133 municipal/provincial supervisors and 11 national facilitators. From October 2019-March 2020 a sample of 76.6% of health facilities (276/360), having 80% of malaria cases in the region, were visited to check for patient and antimalarial stock records accuracy and an average of 3 health providers were observed on MCM. Performance results were available immediately for feedback to facilities and linked to the MOH central DHIS2 server. Overall, facilities' highest scores were for malaria diagnosis using RDTs (performance score range: 85.4%-92.7%, mean: 89.5%), while facilities' lowest scores were for MCM in children (performance score range: 60.0%-79.6%, mean: 72.1%). A total of 142 facilities (51.4%) were identified as high priority for training and follow-up. Information on providers' performance became available in real-time to decision-makers at municipal level, to inform prioritization and to develop monthly training and supervision plans for low-performing health units, tailored to address the identified gaps along the continuum of MCM. The use of this digital supervision tool ensured

municipal supervisors provided immediate quality-assured feedback to health providers across 60 high endemic municipalities improving the management of the limited resources available.

292

STUDY FOR PILOTING PROCEDURES FOR RECRUITMENT AND SCREENING AND BUILDING A REGISTRY FOR POTENTIAL RESEARCH PARTICIPANTS FOR FUTURE MALARIA CLINICAL TRIALS: EXPERIENCE FROM BIOKO ISLAND MALARIA ELIMINATION PROGRAM CLINICAL RESEARCH CENTER, EQUATORIAL GUINEA

Maria Silvia A. Lopez Mikue[1], Vicente Urbano Nsue Ndong[1], Ali Hamad[2], Thabit Mbaga[2], Beltran Ekua Ntutumu[1], Federico Comsil Chochi[3], Wonder Philip Phiri[3], Marta Alene Owono Eyang[1], Juan Carlos Momo[3], Martin Eka Ondo[3], Genaro Nsue Nguema[1], Antonio Enrique Ngua Sama Roca[3], Matilde Riloha Rivas[4], Gertrudis Owono Bidjimi[1], Fortunata Lobede Mochomuemue[1], Ines Toichoa[3], Judith Mitogo[3], Jose Enrique Lima[3], Julia Yumbe Baka[3], Jose Raso[3], Mariano Obiang Obono[1], Marcos Mbulito Iyanga[3], Olivier Tresor[3], Raul Chuquiyauri[3], Preston Church[5], Gabriel Mba[3], Peter Billingsley[5], Restituto Moka Bila[3], Jeremias Nzamio[3], Godwin Fuseini[3], Guillermo Garcia[3], Maximilian Mpina[6], Elizabeth Nyakarungu[2], Claudia Daubenberger[6], Stephen L. Hoffman[5], Salim Abdulla[2], Thomas Richie[5], Kamaka Ramadhani Kassim[7], Said Jongo[2]

[1]*Ministry of Health and Social Welfare of Equatorial Guinea, Malabo, Equatorial Guinea,* [2]*Ifakara Health Institude, Dar es Salam, United Republic of Tanzania,* [3]*Medical Care Development International, Malabo, Equatorial Guinea,* [4]*Ministry of health and Social Welfare, Malabo, Equatorial Guinea,* [5]*Sanaria Inc., Rockville, MD, United States,* [6]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [7]*Ifakara Health Institute, Dar es salaam, United Republic of Tanzania*

Malaria Control strategies have resulted in a successful reduction of malaria transmission in Africa and raise optimism for the elimination and eradication of malaria through the use of a vaccine. To date, there is no registered vaccine to provide any level of protection against malaria. To further clinical development of the Sanaria® PfSPZ vaccine, there is a plan to conduct a phase III clinical trial in Equatorial Guinea. The success for this plan depends on the ability to foresee challenges in the process of recruitment of study participants. Failure of meeting the recruitment targets will be the a significant barrier to effective planning and implementation. To ensure timely recruitment, the primary objectives for the present study are to pilot Recruitment and Screening procedures as well as to build a Registry for Potential Research Participants. The present study begun in September 2019 after receiving protocol approval from the local and international ethics committees and is currently ongoing in Bioko Island, Equatorial Guinea. This cross-section assessment will screen approximately 3500 individuals, including children, adolescents, and adults aged 1.5 to 50 years old living in selected areas of Bioko Island with high malaria transmission. The target is to include approximately 2500 potential participants in the clinical trial registry of upcoming clinical trials in Equatorial Guinea. Potential research participants will be identified using household questionnaire and invited for the screening procedures at the recruitment venue established close to their locality with further screening at study clinics. Challenges and mitigation processes will be described. Descriptive statistics will be used to compute the number of households visited, number of individuals attending town hall meetings, number of individuals providing informed consent, number of individuals meeting eligibility criteria suitable for future vaccine trials, and number of individuals willing to be placed into the registry. The final monitored data is expected to be available in July 2020.

293

RELEVANCE OF DIFFERENT RECRUITMENT APPROACHES PROCEDURES DURING EQUATORIAL GUINEA MALARIA VACCINE INITIATIVE

Juan Carlos Momo Besaha[1], Raul Chuquiyauri[2], Martin Eka[1], Said Jongo[3], Vicente Urbano Nsue Ndong[1], Peter Billingsley[4], Matilde Riloha Rivas[1], Salim Abdulla[3], Kamaka Ramadhani Kassim[3]

[1]*Ministry of Health and Social Welfare, Malabo, Equatorial Guinea,* [2]*Medical Care Development International, Malabo, Equatorial Guinea,* [3]*Ifakara Health Institute, Dar es salaam, United Republic of Tanzania,* [4]*SANARIA INC., Rokville, MD, United States*

Randomized controlled trials are widely recognized as the most robust study design for clinical research. One of the greatest challenges in the conduct of clinical trials is participant recruitment. This process begins with protocol development and ends when the desired sample is obtained through the successful accrual of subjects. The Strategy used during this process can determine the efforts and time that need to be invested. The Equatorial Guinea Malaria Vaccine Initiative (EGMVI) project employed different recruitment approaches to recruit clinical trial subjects since its commencement in 2014. The project has been taken different steps including the adjustment of exclusion/inclusion and using of community approach to improve the rate of recruitment in the clinical trial. There is no standardize study has been done to assess the effectiveness of various recruitment strategies implemented by the EGMVI project. Our aim is to describe the different recruitment approaches and assess the effectiveness of these approaches that have been implemented during the EGMVI clinical studies (EGSPZV2, EGSPZV3 and EGRESPAR). This study will help to identify the best approaches to employ in an environment where clinical trials are new to optimum recruitment. It will also help for future studies to decide the more convenient approach base on the protocol design and the available trial resources. In this study, the differences between all steps of recruitment will be described. The proportion of failures will be calculated in each step of the recruitment process. Further, the logistic effort will be estimated in the different approaches to recruitment. The data will be available and analyzed in august, before the 2020 ASTMH Annual Meeting.

294

UNDERSTANDING OF INFORMATION SHEET AND THE COMMON QUESTIONS ASKED DURING THE CONSENTING PROCESS FOR FUTURE MALARIA VACCINE CLINICAL TRIAL AMONG BIOKO ISLAND POPULATION

Concepcion Moyong Mba Andeme[1], Vicente Urbano Nsue Ndong[2], Maria Silvia Angue Lopez Mikue[2], Said Jongo[3], Thabit Mbaga[3], Beltran Ekua Ntutumu[2], Ali Hamad[3], Wonder Philip Phiri[1], Julia Yumbe Baka[1], Ines Toichoa[1], Olivier Tresor[1], Godwin Fuseini[1], Guillermo Garcia[1], Genaro Nsue[2], Juan Carlos Momo[2], Martin Eka Ondo Mangue[2], Maximillian Mpina[4], Elizabeth Nyakarungu[3], Jose Raso[2], Matilde Riloha Rivas[2], Raul Chuquiyauri[1], Preston Church[5], Peter Billingsley[5], Thomas Richie[5], Stephen L. Hoffman[5], Claudia Daubenberger[4], Salim Abdulla[3], Kamaka Ramadhani Kassim[3]

[1]*Medical Care Development International, Malabo, Equatorial Guinea,* [2]*Ministry of Health and Social Welfare, Malabo, Equatorial Guinea,* [3]*Ifakara Health Institute, Dar es salaam, United Republic of Tanzania,* [4]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [5]*Sanaria Inc, Rockville, MD, United States*

Informed consent is one of the widely accepted legal, ethical, and regulatory requirements for all clinical research involving human participants. Consent has two specific goals in clinical research :(i) to respect and promote a participant´s autonomy, and (ii) to guard participants from harm. It's the method where a participant´s informed about all the important aspects of the trial for them to make an informed decision and provide voluntary authorization of the participation or refusal of the participant. The quality of consent in clinical research is decided by the extent to which participants understand the content of the informed

consent. Understanding of informed consent plays a pivotal role in clinical research because it directly affects how ethical principles of respect and autonomy are applied in practice. Understanding of consent form implies that research participants are ready to comprehend the knowledge provided and appreciate its relevance to their personal situations. Little is known about the study participants' understanding of the knowledge given during the consent process in African countries including Equatorial Guinea. Therefore, there is a need to assess the extent of understanding of information sheets during the consenting process of malaria vaccine clinical trials among the Bioko Island population in Equatorial Guinea. The design of the study is cross-sectional. This study will do an analysis of all informed consent forms done during the implementation of the study with the title 'Pilot Study to Optimize Recruitment and Screening Procedures for Future Clinical Trials and to create a Registry of Potential Research Participants on Bioko Island, Equatorial Guinea. The study population is expected to be 3500 adult individuals aged 18 to 50 and the parent or guardian of the children age 1.8 to 17 years old. Descriptive analysis will be used to assess the level of the understanding of the participant and identify the common questions documented during the informed consent process. Data will be presented by tables and charts. The study started in September 2019 and final monitored data will be available in July 2020.

295

NORMAL LABORATORY REFERENCE INTERVALS AMONG HEALTHY CHILDREN, ADOLESCENTS, AND ADULTS AGED 1.5 TO 50 YEARS OLD SCREENED FOR A MALARIA CLINICAL TRIAL IN BIOKO ISLAND, EQUATORIAL GUINEA

Genaro Nsue Nguema Okomo[1], Gertrudis owono[1], Ali Hamad[2], Kamaka Kassim[2], Guillermo Garcia[3], Maria Silvia Lopez Mikue[1], Elizabeth Nyakarungu[4], Matilde Riloha Rivas[1], Thomas Richie[5], Claudia Daubenberger[6], Said Jongo[2]

[1]*Ministry of Health and Social Welfare, Malabo, Equatorial Guinea,* [2]*Ifakara Health Institute, Bagamoyo, United Republic of Tanzania,* [3]*Medical Care Development International, Malabo, Equatorial Guinea,* [4]*Ifakara Health Institute, Dar es salaam, United Republic of Tanzania,* [5]*Sanaria Inc, Rockville, MD, United States,* [6]*Swiss Tropical and Public Health Institute, Basel, Swaziland*

In clinical trials, relevant reference ranges are required for the correct interpretation of standard laboratory test results from the target population. In addition, reference ranges are necessary to accurately assess possible adverse events observed during clinical trials. Most clinically healthy participants have been excluded from several clinical trials in Africa because haematological and biochemical laboratory parameters are classified as abnormal. This unnecessary exclusion of potential participants usually results in an increased cost of study recruitment to achieve the target sample size. To overcome these challenges, accurate reference ranges, derived locally, need to be established for the target population. Objectives were, to establish normal laboratory range parameters for complete blood count (CBC) and clinical biochemistry, in children, adolescents and adults living in Equatorial Guinea, determine the effect of sociodemographic and nutritional factors on hematological parameters, to know the relationship between the educational level of participants and haematological references and to compare the values obtained locally with the international references currently in use. A cross-sectional study including approximately 1200 Children was used, adolescents, and adults aged 1.5 to 50 years old living in selected areas of Bioko Island with high malaria transmission. Laboratory reference values shall be estimated using non-parametric methods according to sex. The mean (with 95% confidence interval), the median, the range and the 2.5 and 97.5 percentiles. The mean and the corresponding 95% CI will be calculated. A non-parametric test of the Wilcoxon sum score shall be performed to check the gender differences of each analyte. The reference interval will be estimated by a specific percentage (in this case 95%) of the values for a population from which the reference subjects were extracted. A univariate and multivariate analysis of the sociodemographic and nutritional factors as well as the educational level influenced by the normal laboratory reference ranges will be performed.

296

BUILDING HUMAN RESOURCES CAPACITY FOR CLINICAL RESEARCH IN EQUATORIAL GUINEA

Celestina Panades Zamora[1], Juan Carlos Momo Besaha[2], Said Jongo[3], Matilde Riloha Rivas[2], Guillermo A. Garcia[4], Peter Billingsley[5], Salim Abdulla[3]

[1]*Medical Care Development International, Malabo, Equatorial Guinea,* [2]*Ministry of Health and Social Welfare, Malabo, Equatorial Guinea,* [3]*Ifakara Health Institute, Dar es salaam, United Republic of Tanzania,* [4]*Medical Care Development International, Silver Spring, MD, United States,* [5]*SANARIA INC., Rockville, MD, United States*

Capacity development is the process of enabling individuals or systems to recognize and solve their own problems, make informed choices, define priorities and plan their futures in a sustainable manner. This effort may be targeted to specific individuals, institutions, organizations, communities or entire nations. Similarly, it may be targeted to specific tasks and needs or in broad terms at the overall strengthening of systems. In general, clinical research human resource capacity building refers to programs aimed at enhancing knowledge of researchers and people involved in clinical trials to conduct clinical research. Until 2014, no clinical trials had been conducted in Equatorial Guinea. This means that the country did not have staff with experience in clinical research. From 2014 to date, studies have been implemented involving local staff. Great efforts have been made to draw up a plan for training and empowering local staff. A formal training plan was put in place with different nuances and approaches. Similarly, a plan to empower local staff has been put in place. No standardized evaluation has been established to know the extent of the achievements being made and to measure the progress of the training and empowerment plan in a systematic way. Our aim is to describe the training and empowerment plan, to evaluate the progress made over time and to identify the weaknesses found during this time. The different steps taken in the formal training plan will be analyzed, the proportion of staff trained in different approaches will be calculated and the proportion of people promoted to decision-making positions in each training approach will be calculated.

297

PREVALENCE AND CHARACTERIZATION OF COMMON HEALTH CONDITIONS AMONG INDIVIDUALS LIVING IN SELECTED AREAS OF BIOKO ISLAND WITH HIGH MALARIA TRANSMISSION WHO SCREENED FOR FUTURE MALARIA VACCINE CLINICAL TRIAL IN BIOKO ISLAND, EQUATORIAL GUINEA

Fortunata L. Mochomuemue[1], Manuel Francisco Mfumi[1], Vicente Urbano Nsue Ndong[1], Maria Silvia Angue Lopez Mikue[1], Thabit Mbaga[2], Wonder Philip Phiri[3], Guillermo Garcia[3], Elizabeth Nyakarungu[2], Jose Raso[1], Matilde Riloha Rivas[1], Thomas Richie[4], Claudia Daubenberger[5], said Jongo[2]

[1]*Ministry of Health and Social Welfare, Malabo, Equatorial Guinea,* [2]*Ifakara Health Institute, Dar es salaam, United Republic of Tanzania,* [3]*Medical Care Development International, Malabo, Equatorial Guinea,* [4]*Sanaria Inc, Rockville, MD, United States,* [5]*Swiss Tropical and Public Health Institute, Malabo, Equatorial Guinea*

Characterization of Health condition facing the setting community is an important input to national and international health decision making and planning processes including a clinical trial. The Bioko Island Malaria Elimination Program (BIMEP) is planning a large Phase 3 trial of Sanaria® PfSPZ Vaccine on Bioko Island, to start in 2020, with plans to enroll 2100 research subjects ranging in age from 2 to 50 years. Therefore, in this planning stage, there is a need for information on the prevalence of common conditions for both communicable and non-communicable diseases to be known in order to provide a picture of the health status of the target community. Also, it will give a context for health issues that may arise in future clinical trials in this community. To describe the common health conditions among individuals living in selected areas of Bioko

Island with high malaria transmission who screened for future malaria vaccine clinical trial in Bioko Island, Equatorial Guinea. The study design is a descriptive cross-sectional study. The data will be obtained from the clinical data-set of the study entitled Pilot Study to Optimize Recruitment and Screening Procedures for Future Clinical Trials and to Create a Registry of Potential Research Participants on Bioko Island, Equatorial Guinea. The pilot study is expected to recruit 3500 healthy males and females aged 18 months to 50 years old living in selected areas of Bioko Island with high malaria transmission. Descriptive analysis will be used to describe various health conditions such as positive HIV, HBV or HCV or conditions such as anemia, hypertension, and splenomegaly in individuals who deem themselves to be healthy living in Bioko Island. The statistical analysis will be done by SPSS software. The study started in September 2019 and final monitored data is expected to be available in July 2020.

298

THE IMPACT OF COVID-19 PANDEMIC TO THE PLANNED AND ONGOING CLINICAL RESEARCH AND CLINICAL RESEARCH SITE MITIGATION STRATEGIES IN BIOKO ISLAND, EQUATORIAL GUINEA IN YEAR, 2020

Marta Alene Owono Eyang[1], Vicente Urbano Nsue Ndong[1], Maria Silvia Angue Lopez Mikue[1], Wonder Philip Phiri[2], Juan Carlos Momo[1], Peter Billingsley[3], Claudia Daubenberger[4], Kamaka Kassim[5], said Jongo[5]

[1]*Ministry of Health and Social Welfare, Malabo, Equatorial Guinea,* [2]*Medical Care Development International, Malabo, Equatorial Guinea,* [3]*Sanaria Inc, Basel, MD, United States,* [4]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [5]*Ifakara Health Institute, Dar es salaam, United Republic of Tanzania*

The world has been impacted by the 2019 novel coronavirus SARS-CoV-2 and the resulting disease, "COVID-19". The current containment efforts in Equatorial Guinea and the world at large have disrupted the implementation of ongoing and planned clinical Research at The Bioko Island Malaria Elimination Program (BIMEP). BIMEP executes the study entitled Pilot Study to Optimize Recruitment and Screening Procedures for Future Clinical Trials and to Create a Registry of Potential Research Participants on Bioko Island, Equatorial Guinea". Also, BIMEP) has a plan to conduct a large Phase 3 trial of Sanaria® PfSPZ Vaccine on Bioko Island, to start in 2020, with plans to enroll 2100 research subjects ranging in age from 2 to 50 years. The clinical trial execution could significantly endanger the safety of study participants and study staff by creating opportunities for SARS-CoV-2 transmission, seeding communities with infected individuals as a result of encounters with clinical staff or other participants during research activities. Therefore, there is a need to documents how the Bioko Island Malaria Elimination Program impacted and mitigation strategies to minimize the effect of COVID-19 pandemic. To describe the impact of COVID-19 pandemic on the planned and ongoing clinical research and clinical research site mitigation strategies in Bioko Island, Equatorial Guinea. The study will use qualitative techniques to analyze study documents and reports to gain a broad understanding of the situation. The data will be collected through a desk review technique and qualitative methodology will be utilized for analysis. The impact of the COVID-19 pandemic will be described in terms of the financial impact on the program, monitoring of participant safety, Loss of experienced staff and delay of clinical trial schedule. The information is expected to be analyzed and available for the ASTMH Annual Meeting before August 2020.

299

SAFETY OF PFSPZ-CVAC (PYR) VACCINATION AGAINST *PLASMODIUM FALCIPARUM* IN HEALTHY ADULTS IN BANCOUMANA, MALI

Mahamadoun H. Maiga[1], David M. Cook[2], Issaka Sagara[1], Kourane Sissoko[1], Sibiri Sissoko[1], Amatigue Zeguime[1], Seydou Sankare[1], Sadio Diarra[1], Mamady Kone[1], Sintry Sanogo[1], Moussa B. Kanoute[1], Sara Healy[2], Jacquelyn Lane[2], Alemush Imeru[2], Emily Higbee[2], Mahamadou S. Sissoko[1], Peter Billingsley[3], Kim L. Sim[3], Thomas L. Richie[3], Steven L. Hoffman[3], Patrick E. Duffy[2]

[1]*Malaria Research and Training Center, Mali-NIAID ICER, University of Science, Techniques and Technologies of Bamako, Bamako, Mali,* [2]*Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States,* [3]*Sanaria Inc, Rockville, MD, United States*

An ongoing randomized double-blind placebo-controlled study in Mali is evaluating the safety and vaccine efficacy (VE) against naturally transmitted *Plasmodium falciparum* (Pf) malaria infection of 3 doses of $4.0x10^5$ PfSPZ of Sanaria PfSPZ Challenge (aseptic, purified, cryopreserved , infectious Pf sporozoites[SPZ]) administered by direct venous inoculation (DVI) at 0, 4, and 8 weeks combined with oral administration of a chemoprophylactic drug, either pyrimethamine (PYR) or chloroquine (CQ), in a vaccination approach termed PfSPZ-CVac (Chemoprophylaxis Vaccination). Both PfSPZ-CVac (CQ) and (PYR) have induced long-lived sterile immunity to homologous and recently to heterologous controlled human malaria infection. We are first exploring safety and VE of PfSPZ-CVac (PYR) in 240 Malian adults randomized to 4 arms receiving 3 DVI injections with PfSPZ Challenge or normal saline every 4 weeks. Arms V_0 (active, n=90) and P_0 (control, n=54) received 75mg of PYR on the same day as each injection (day 0) while Arms V_{2+3} (active, n=60) and P_{2+3} (control, n=36) received 75mg of PYR on days 2 and 3. Participants received artemether/lumefantrine 2 weeks prior to the 1st and 3rd injections to clear any naturally acquired Pf infections. Post vaccination, participants were monitored for Pf by thick blood smear (TBS) every two weeks for 26 weeks. Vaccinations were safe and well tolerated. Both the more practical day 0 PYR regimen and the days 2 + 3 PYR regimen prevented asexual Pf parasitemia induced by PfSPZ Challenge, confirming results from an earlier pilot phase. There was a single SAE which was unrelated to study procedures (intestinal volvulus). Of the 1001 total adverse events (AEs), 11 (1.1%) were grade 3. 3 of these, all neutropenia, were considered possibly related. The other grade 3 AEs (hypertension, paronychia, joint dislocation) were not related. 51% of total AEs were grade 2, most being lab abnormalities or rhinitis that were unrelated. 112 first Pf infections in unique individuals were documented by TBS, 65/144 (45.1%) in Arms V_0 / P_0 and 47/96 (49.0%) in Arms V_{2+3} / P_{2+3}. The study remains blinded and the CQ arms will be enrolled at a later date.

300

SAFETY AND EFFICACY OF IMMUNIZATION WITH RADIATION-ATTENUATED *PLASMODIUM FALCIPARUM* SPOROZOITE (PFSPZ) VACCINE IN 1-12-YEAR-OLD GABONESE IN LAMBARÉNÉ

Selidji Todagbe Agnandji[1], Matthew B.B. McCall[2], Armel Vivien Mbouna[1], Ayodele Alabi[1], Anita Lumeka Kabwende[1], Paulin N Essone[1], Thomas L Richie[3], L.W. Preston Church[3], B. Kim Lee Sim[3], Stephen L. Hoffman[3], Peter G Kremsner[4], Benjamin Mordmüller[4]

[1]*CERMEL, Lambaréné, Gabon,* [2]*Department of medical microbiology, Radboud University medical center,, Nijmegen, Netherlands,* [3]*Sanaria Inc, Rockville, MD, United States,* [4]*Institut für Tropenmedizin, Universitätsklinikum, Tuebingen, Germany*

In the quest for malaria elimination and eradication, a vaccine is an essential and complementary tool to the current preventive and curative interventions. Whole *Plasmodium falciparum* (Pf) sporozoite (SPZ)-based vaccines have been assessed worldwide and show up to 100% protection in controlled human malaria infections. In 3 trials in African

adults, efficacies against naturally acquired Pf infection of 52%, 51% and 47% have been demonstrated during 6 months, and in one of these trials shown to be sustained for 18 mo. Compared to subunit vaccines, PfSPZ vaccines have the advantages of a diverse antigenic spectrum, translating conceptually into broad protection. Immunization by direct venous inoculation (DVI) with PfSPZ improves the breadth and magnitude of vaccine-induced immune responses, and this is essential to reach high efficacy with additional benefits of greater tolerability and safety, since no adjuvant is required. We performed a randomized, double blind, placebo-controlled Phase 2 trial to assess the safety, tolerability, immunogenicity and vaccine efficacy (VE) of DVI administration of the radiation-attenuated PfSPZ Vaccine at a dose of $9x10^5$ PfSPZ on Days 1, 8 and 29 in 200 children aged 1-12 years living in Lambaréné, Gabon. It is the first VE trial of PfSPZ Vaccine in the most important age-group for malaria control. Respectively 64, 99 and 37 children aged 7-12, 3-6 and 1-2 years were enrolled between July 2018 and March 2019 and randomly allocated at a 2:1 ratio to received PfSPZ Vaccine or normal saline placebo. 193 (97%) children received all 3 vaccinations, and 89% of those were given exactly as scheduled. We will report safety, tolerability and VE of PfSPZ Vaccine assessed between two weeks and 26 weeks after the third immunization against naturally acquired Pf infection detected by passive and active (every two weeks from 2 weeks (14±2 days) until 26 weeks after the final immunization) follow up using thick blood smear microscopy. 84 malaria episodes have been observed through April 2020; 40 within the time-window for primary analysis. This incidence rate will allow detection of ~60% VE with a power of 80% and alpha of 5% (2-tailed).

301

MANUFACTURING PURIFIED, CRYOPRESERVED *PLASMODIUM VIVAX* SPOROZOITES FOR CONTROLLED HUMAN MALARIA INFECTION

Sumana Chakravarty, Natasha KC, Urvashi Rai, Abraham Eappen, Tao Li, MingLin Li, Anita Manoj, B. Kim Lee Sim, Stephen L. Hoffman

Sanaria Inc., Rockville, MD, United States

*Plasmodium vivax (*Pv), is the most prevalent cause of malaria-associated morbidity outside of Africa. However, significant challenges in propagating the life cycle of Pv *in vitro* limit the scope of antigen and drug discovery, and availability of suitable models for interventional testing against this pathogen. Our goal is to develop a Pv vaccine using a whole-sporozoite (SPZ) based approach similar to PfSPZ, that prevents all blood stage infections with Pv, and also produce quality-controlled stocks of cryopreserved PvSPZ to promote well-controlled, reproducible *in vitro* and *in vivo* studies in Pv including CHMI. To this end we have, 1) Demonstrated the capacity to repeatedly manufacture vialed, cryopreserved PvSPZ from mosquitoes fed on blood from *Saimiri boliviensis* (Sb), vialing as many as 80 million PvSPZ from 2000 mosquitoes in 1 day; 2) Developed assays to test and characterize the PvSPZ for infectivity *in vitro* in traditional monolayer formats over 3-6 days and in micro-patterned co-cultured primary human hepatocytes over 12-21 days; 3) Successfully tested functional activity of drugs on inhibiting liver stage parasite development *in vitro;* 4) Provided proof of infectivity *in vivo* in NHPs; 5) Recently standardized a method to propagate Pv in Sb that were rendered and maintained as a specific pathogen free (SPF) colony at the only primate facility and source of Sb bred in captivity in the US; and 6) have now vialed aseptic, purified, infectious PvSPZ generated in aseptic mosquitoes using Pv-infected blood from the SPF animals. With an overall manufacturing process analogous to production of aseptic purified, cryopreserved PfSPZ, the PvSPZ products of 2 pilot runs conformed to all in-process, asepticity, *in vitro* potency, and release criteria, constituting a significant milestone towards regulatory compliance for human use. This enabling technology is intended to support the development and testing of anti-Pv drugs and vaccines in CHMIs world-wide, and also form the basis of a powerful vaccine approach to preventing Pv malaria when administered with anti-malarial chemoprophylaxis, the PvSPZ chemoprophylaxis vaccine (PvSPZ-CVac).

302

COMPARABLE SAFETY PROFILES OF PFSPZ VACCINE AND NORMAL SALINE ADMINISTERED BY DIRECT VENOUS INOCULATION IN ADULTS AND CHILDREN - A META-ANALYSIS OF 17 RANDOMIZED CONTROLLED CLINICAL TRIALS

LW Preston Church[1], Pouria Riyahi[1], the International PfSPZ (iPFSPZ) Consortium

[1]*Sanaria Inc, Rockville, MD, United States*

The safety of Sanaria® PfSPZ Vaccine has been demonstrated in 17 phase 1 and 2 randomized, controlled trials (RCT) in adults in the US and EU and in infants, children and adults at multiple sites in sub-Saharan Africa. The vaccine is composed of radiation-attenuated, aseptic, purified, cryopreserved *Plasmodium falciparum* (Pf) sporozoites (SPZ) administered by direct venous inoculation (DVI) with normal saline (NS) as the comparator. Because the size of each RCT (18 to 336 subjects) limited the power of each trial, we analyzed the available safety data using meta-analysis. Data from all completed RCTs were included as either age > 18 years (n=497) or age 5 months to 18 years (n=641). Any subject receiving at least one dose of PfSPZ Vaccine or NS was included. A fixed-effect model was used to study vaccine safety, and data evaluated using heterogeneity and I^2 statistic. In all studies PfSPZ vaccine was well-tolerated - 130 of 794 (16.4%) in the vaccinees and 49 of 344 (14.2%) in the controls reported systemic solicited adverse events (AE) (p=0.38). The fixed-effect risk ratio (RR) for all vaccine-related adverse events reported was 0.99 (95% confidence intervals (CIs) 0.77-1.27) for younger subjects (5 months to 18 years old) and 1.19 (95%CIs 0.82-1.72) in adults older than 18 years. Headache (29%), fever (27%) and fatigue (9%), were the most frequent AEs. In subjects 5 months to 18 years, there was no significant difference in risk between vaccinees and controls for any AE (for fever, RR: 1.1; 95%CIs 0.82-1.51). In adults there was a significant increase in vaccine-related headaches in vaccinees as compared to controls (RR: 1.43; 95%CIs 1.09-1.82), but not for any other AE (for fever, RR: 0.75; 95%CIs 0.42-1.32). PfSPZ vaccine was safe and well-tolerated in adult and pediatric subjects, with headache as the only solicited AE that was more frequent in vaccinated adult subjects as compared to controls and no differences in AEs in pediatric subjects. Overall, adverse events were not different between PfSPZ Vaccine and NS.

303

THE CREATION OF NOVEL WHOLE SPOROZOITE VACCINES FOR MALARIA BY DOMINANT-NEGATIVE MUTATION

Ashley Michael Vaughan, Charlie Jennison, Janna Armstrong, Stefan Kappe

Seattle Children's Research Institute, Seattle, WA, United States

Genetically attenuated malaria parasite vaccines that arrest during liver stage development are powerful immunogens and in human clinical trials provide complete protection from controlled malaria human infection. To date, genetic attenuation by gene deletion has been the only reliable means to generate parasites that arrest in the liver. However, the introduction of dominant-negative mutations has been used throughout biology to assess genotype/phenotype relationships. We thus investigated whether the expression of dominant-negative versions of parasite proteins during liver stage development could attenuate the parasite. We reasoned that expressing dominant-negative mutants under liver stage-specific promoters would not affect any other stage of the life cycle. We targeted parasite processes within the essential mitochondria and apicoplast organelles of the parasite. Members of the caseinolytic protease (Clp) superfamily are involved in proteolytic processing essential for the homeostasis of these organelles, akin to proteasome-related degradation in the cytoplasm. Specifically, the oligomeric ClpQ threonine protease traffics to the mitochondria and the oligomeric ClpP serine protease traffics to the apicoplast. We hypothesized that attenuation could be achieved by expression of dominant-negative protease deficient ClpQ and ClpP, respectively. We used rodent malaria *Plasmodium yoelii* for proof-

of-concept. We replaced the strong liver stage-specific *LISP2* locus by either mutant ClpQ (mClpQ) or mutant ClpP (mClpP). We injected 75,000 sporozoites into groups of mice for the four genotypes - *P. yoelii* wildtype, *lisp2*$^-$, *lisp2*$^-$/mClpQ and *lips2*$^-$/mClpP. Time to blood stage patency was analyzed for each group of mice. Strikingly, *lisp2*$^-$/mClpQ and *lips2*$^-$/mClpP parasites were more severely attenuated in the liver compared to *lisp2*$^-$ alone, providing evidence that the dominant-negative approach can be used for attenuation. Further studies are now underway to create completely attenuated human *P. falciparum* dominant-negative mutant parasites for vaccine studies.

304

MATHEMATICAL MODELING OF ANOPHELES MOSQUITOES' BREEDING SITES TO SUSTAIN EFFICIENT VECTOR CONTROL

Toheeb Babatunde Ibrahim[1], Kristan A. Schneider[2], Martin Eichner[1]

[1]*University of Tuebingen, Tuebingen, Germany,* [2]*Hochschule University of Applied Science, Mittweida, Germany*

Vector control interventions must properly account for the entomological characteristics of the targeted species to be efficient. Proper understanding of the breeding habits and sites of a targeted mosquito vector can facilitate environmentally sustainable and cost-efficient vector control. This, however, is a factor not properly addressed yet in predictive models. An *Anopheles* (An.) mosquitoes' breeding site can form when rain falls and mosquitoes find water puddles to lay their eggs. Over time, other organisms cohabit the breeding site with the mosquito larvae, e.g., fish, tadpoles, etc. They contribute to the depletion of nutrients in the breeding site and to the mortality of mosquito larvae. A biological concept describing the dynamics of An. breeding sites was incorporated into a system of ordinary differential equations. The model accounts for competition for nutrients among larvae, which results in delayed maturation of larvae. Furthermore, we included i) organisms that cohabit the breeding site, e.g., larvivorous fish, ii) the effects of larvicides, iii) larval competition, and iv) the effect of insecticide treated bed-nets (ITNs) as a control control measure. Mosquitoes are supposed to always find a breeding site to oviposit and the dynamics in finding a site to oviposit is not explicitly studied (only the average time for mosquitoes to initiate oviposition is considered). Mating was not explicitly modeled, since mating behaviour is not well understood and only one mating is sufficient for a female mosquito to fertilise its lifetime eggs. The systems' equilibrium point and the mosquito populations' basic reproduction number, i.e. the number of female mosquitoes arising from one female mosquito without control, are calculated analytically. We simulated the model using realistic parameter values obtained from published field and laboratory observations, to describe the population sizes of the different mosquito stages. Finally, the effects of mosquito control interventions were studied. Extensions of our model to incorporate feeding habits of mosquitoes and malaria transmission are planned in the future.

305

FINE SCALE ELUCIDATION OF THE SPATIAL EFFECTS OF HOUSEHOLDS AND CLIMATIC FACTORS ON MOSQUITO ABUNDANCE, BUILDING A CASE FOR FURTHER PREDICTIVE MODEL ENHANCEMENTS

Christabellah Namugenyi[1], Martin Lukindu[2], Krystal Birungi[3], Paul Mabuka[3], Agapitus Kato[3], Louis Mukwaya[3], JohnBosco Asiimwe[1], Leonard K. Atuhaire[1], Jonathan Kayondo[3]

[1]*Makerere University, Kampala, Uganda,* [2]*University of Notre Dame, Notre Dame, IN, United States,* [3]*Uganda Virus Research Institute, Entebbe, Uganda*

Vector control is still the most popular strategy in reducing malaria prevalence in sub-Saharan Africa, a region that overwhelmingly shoulders the global malaria burden. A thorough understanding of mosquito population dynamics is crucial for development of novel vector control tools. Weather variability directly influences mosquito density seasonal variations hence climatic based models are often used for its prediction. Unfortunately, current models are restrictive in that no attention is accorded to spatial relations within immediate vicinity of sampled households which may have a direct/indirect impact on mosquito density. In this study we used a Generalized Spatial Panel Random Effects (GSPRE) model to examine the effect of climatic factors and spatial relations on household mosquito abundance in a high mosquito density village, in eastern Uganda. Previously collected data was used for this study. *Anopheles gambiae* mosquitoes, weather variables, and spatial attributes were collected from 66 geo-referenced households from September2017-July2019. Spatial relations were summarized using a spatial weights matrix that considered a household within an arc distance radius of 750 meters to be in neighborhood of a sampled household. Our model results showed a significant positive correlation between mosquito density and the following spatial relations and attributes i) mosquitoes in neighboring households (Coef =1.015 SE=0.125) ii) number of house occupants (Coef = 1.822 SE= 0.488). Expectedly a positive correlation was observed with weather variables; rainfall (Coef =1.125 SE=0.302) and temperature (Coef =4.358 SE=1.499). However, sunlight was negatively correlated to mosquito counts (Coef = -0.769 SE=0.317). Our study further stresses the need to incorporate spatial relations in sampling sites in order to accurately predict mosquito densities in malaria endemic areas so as to make more informed decisions in vector interventions. We have successfully shown that mosquitoes in houses within close proximity of a sampled household have a significant effect on its mosquito counts hence refining the accuracy of climatic models.

306

CREATING MOSQUITO-FREE OUTDOOR SPACES USING TRANSFLUTHRIN-TREATED CHAIRS & RIBBONS

John Paliga Masalu, Marceline Finda, Gerry F. Killeen, Halfan S. Ngowo, Polius G. Pinda, Fredros O. Okumu: O. Okumu

Ifakara Health Institute, Morogoro, United Republic of Tanzania

Residents of malaria-endemic communities spend several hours outdoors performing different activities, thereby exposing themselves to potentially-infectious mosquitoes. These behaviors compromise effectiveness of long-lasting insecticide-treated nets (LLINs) & indoor residual spraying (IRS). Common peri-domestic spaces were characterized in rural Tanzania, & assessed protective efficacies of transfluthrin-chairs & ribbons, against mosquitoes. Two hundred households were surveyed, & their peri-domestic spaces physically characterized. Protective efficacies of transfluthrin-chairs & ribbons were tested in outdoor environments of 28 households, using exposure-free double net traps. CDC light traps were used to estimate host-seeking mosquito densities within makeshift kitchens. Field-collected *Anopheles arabiensis* & *Anopheles funestus* mosquitoes were exposed underneath the chairs to estimate 24h-mortality. Approximately 52% of houses had verandas. Aside from these verandas, most houses also had peri-domestic spaces (67% of houses with verandas & 94% of non-veranda houses). Two-thirds of these spaces were sited under trees, and 34.4% were built-up. The outdoor structures were usually makeshift kitchens having roofs & partial walls. Transfluthrin-treated chairs reduced outdoor-biting *An. arabiensis* densities by 70-85%, while transfluthrin-treated hessian ribbons fitted to the outdoor kitchens caused 77-81% reduction in the general peri-domestic area. Almost all the field-collected *An. arabiensis* (99.4%) & *An. funestus* (100%) exposed under transfluthrin-treated chairs died. Most houses had actively-used peri-domestic outdoor spaces where exposures to mosquitoes occur. Both the transfluthrin-treated chairs & ribbons reduced outdoor-biting malaria vectors in the peri-domestic spaces, & elicited significant mortality among field-caught malaria vectors. These prototype formats, if developed further, may constitute new options for complementing LLINs & IRS with outdoor protection against malaria & other mosquito-borne diseases in areas with significant peri-domestic activities.

307

BIOSYNTHESIS OF SILVER NANOPARTICLES FROM *OCIMUM BASILICUM*; A NATURAL LARVICIDE AGAINST *ANOPHELES GAMBIAE*

Agnes Ntoumba, Francois Eya'ane Meva, Loick Pradel Kojom, Wolfgang Eyisap Ekoko1, Jerson Mekoulou Ndongo, Philippe Belle Ebanda Kedi, Leopold Gustave Lehman

University of Douala, Douala, Cameroon

Chemical insecticides are still used now for the vector control of parasitic diseases although there are dangerous for human being health. Control of vector is very important in the process of eradication of malaria. Mosquitoes have shown resistance about many insecticides which result is the mutation of them. The fight against adult mosquitoes must be synchronized with the decreasing too number of larvae also it's important to find the new tools which are safe and no toxics for our health. Many areas are explored like a green synthesis which uses plants extract to obtain a natural larvicide. We investigate the efficacy of *Ocimum basilicum*; fresh leaves extract have been used to synthesize silver nanoparticles and this solution has been tested on *Anopheles gambiae* larvae as stipulated by standard methods for testing toxicity and susceptibility of mosquito larvae to insecticides of WHO. Those silver nanoparticles have been characterized to determine their absorbance, size, shape, nature, and chemical components by spectrometry, diffractometry and infrared. The number of larvae dead was recorded after 24 and 48 hours, the rate of mortality and the LC at 50% and 90% was calculated. This method could be innovative in green nanotechnology and in Entomology, very cheap, safe and eco-friendly.

308

CONTRIBUTION OF NON-HUMAN BLOOD MEAL SOURCES IN THE ECOLOGY OF MALARIA VECTORS IN LAKE VICTORIA, TANZANIA

Doris Mangalu, Lucy Bernard, Alphaxard Manjurano, Eric Lyimo, Charles Kakilla, Karen Nelwin

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

Female *Anopheles* mosquitoes are main malaria vectors for spreading *Plasmodium* parasites through taking of a blood meal from an infected to an uninfected person. *Plasmodium* sporozoite transmission stage is critical in the parasite life-cycle progression serving as an essential target for prophylactic drugs and vaccines. The biting and resting behavior of the vectors on the other hand influence their susceptibility to current vector control tools. For example, species that preferentially bite and rest indoors are amenable to both indoor residual spraying and to the use of insecticidal treated nets. The Lake Zone region of Tanzania has one of the highest malaria burdens. We identified four areas for entomological surveillance to understand the biting preference of the local vector species. Two of them have houses sprayed with Clothianidin insecticide and the other two are unsprayed. Methods of mosquito collection included use of Prokopack aspirators indoors; clay pots outdoors and bottle rotators for both indoors and outdoors. Collection from these sites was for seven months and samples were analyzed using ELISA method to detect blood meal source and PCR for mosquito speciation. Results from the analysis showed most mosquitoes preferred mixed blood of human and non-human blood (bovine, goat and/or dog), and non-human blood, while fewer preferred human blood. These mosquito samples also showed the leading specie to be *Anopheles arabiensis* followed by *An. funestus s.s.*, then *An. gambiae s.s.*, and *An. parensis*. In the absence of baseline data before IRS was introduced in 2007, it shows that local vectors have high preference for non-human blood. Moreover, as most of the vector species are *An. arabiensis*, it is not surprising most of the blood meals were mixed. Blood meal data according to sprayed and unsprayed sites showed most mosquitoes preferred non-human blood, then mixed blood, then human blood for sprayed sites; while for unsprayed sites most preferred mixed blood, then human blood, then non-human blood. From the information obtained, there is therefore a need to adopt other control tools that also impact on non-human feeders.

309

QUANTIFYING THE DEMOGRAPHIC AND FITNESS TRAITS OF THE DOMINANT VECTOR OF MALARIA TRANSMISSION, *ANOPHELES FUNESTUS* IN TANZANIA

Halfan S. Ngowo[1], Emmanuel Hape[1], Jason Matthiopoulos[2], Heather Ferguson[2], Fredros Okumu[1]

[1]*Ifakara Health Insititute, Ifakara, United Republic of Tanzania,* [2]*University of Glasgow, Glasgow, United Kingdom*

The behavior of mosquitos plays a key role in their vectorial capacity and the choice of malaria control interventions. While the behavior and life-history of the major African vector group *Anopheles gambiae* has been extensively studied, less is known about the ecology of *Anopheles funestus* due to difficulties in colonizing this species. Given the increasingly prominent role *of An. funestus* in maintaining residual transmission, there is an urgent need to expand our understanding of its ecology. To address this gap, here, we quantified the demography and fitness traits of wild and F1 offspring of *An. funestus* from southern Tanzania during attempted colonization in semi-field conditions. F0 *Anopheles funestus* females were collected from 3 populations near Ifakara, Tanzania and held under laboratory conditions. Eggs produced from these females were used in experiments to assess larval development, blood feeding rates, fecundity and adult survival. These demographic rates generated from F0/F1 will provide useful baseline values for prior parameterization of a Bayesian state-space population model of *An. funestus* which can be fitted to surveillance data from the wild. Wild F0 *An. funestus* collected in the field laid an average of 64.12, 95% CI [58.9, 69.4] eggs in laboratory condition, with eggs to pupae survival rate of 5.87%, 95% CI [3.05, 8.69] and a subsequent life span of 44 days for females and 45 days for males adult mosquitoes. No viable eggs were produced from F1 generation which suggests that, this species is unwilling to mate in insectary conditions. Number of eggs laid by F0 generation were positively associated with their wing size ($p<0.001$). This study provides an understanding of the key fitness parameters of the population dynamics such as adult survival, fecundity and larvae density process that contribute to the population regulation in the larvae and adults stage. A preliminary model of *An. funestus* population dynamics has been developed based on priors generated from the lab study and being adapted to fit in the wild population of *An. funestus* to estimate their fitness and survival parameters.

310

INVESTIGATING THE ROLE OF THE *DOUBLESEX* GENE IN TISSUE DIMORPHISM IN *ANOPHELES GAMBIAE* MOSQUITOES

Nicole M. Page, Maria Giorgalli, Barbara Fasulo, Andrea Crisanti

Imperial College London, London, United Kingdom

Doublesex (*dsx*) is a crucial gene for regulating sex determination in the African malaria vector *Anopheles gambiae*. Knockout out of the female transcript (*dsxF)* in females results in an intersex phenotype causing sterility and male-like morphology. Consequently, *dsx* has become a major focus of research to design novel interventions for vector control. As *Plasmodium* parasites are exclusively transmitted through the bites of infected female mosquitoes, we aimed to investigate the effect of the intersex phenotype on parasite development and transmission, focusing on the tissues involved in vector-parasite interaction. The intersex mosquitoes are unable to bite, and consequently unable to blood-feed. To determine if oocyst development is affected in the absence of *dsxF*, we used artificial infection by injecting ookinetes into the mosquito thorax of male and intersex mosquitoes. To give a more representative view of a typical infection route, we also created transgenic mosquitoes using CRISPR/Cas9 to induce targeted disruption of *dsxF* in the midgut. Restricting the

knockout to the midgut allowed these mosquitoes to be infected via direct blood-feeding as their mouthparts remained unaffected. Although intersex individuals display external male-like morphologies, their midguts and salivary glands resemble those of wild-type females. Artificial infection following ookinetes microinjections showed that *Plasmodium* parasites are able to form sporozoites that later manage to invade the salivary glands in the male and intersex mosquitoes. Midgut-specific knockout of *dsxF* was achieved by introducing indel mutations inside the female-specific exon. Infection of these transgenic females showed that ookinetes were still able to traverse the mutated midgut wall to form oocysts. Although male *Anopheles* mosquitoes lack a shared co-evolutionary pathway with the malaria parasites, they appear to be capable hosts. The barrier of transmission in intersex mosquitoes seems to lie in their inability to bite and sterility rather than any inhibition of the parasite lifecycle.

311

CONTINUED EFFICACY OF PIRIMIPHOS-METHYL (ACTELLIC 300CS) FOR INDOOR RESIDUAL SPRAYING IN AREAS WITH HIGH MALARIA VECTOR RESISTANCE TO PYRETHROIDS IN ZANZIBAR

Khamis A. Haji[1], Bakari O. Khatib[1], Juma H. Mcha[1], Huba H. Ali[1], Makame H. Makame[1], Abdullah S. Ali[1], Faiza B. Abbas[1], Chonge A. Kitojo[2], Naomi Serbantez[2], George Greer[2], Erik J. Reaves[3], Shabbir Lalji[4], Ssanyu S. Nyinondi[4], Bilali Kabula[4]

[1]*Ministry of Health Zanzibar, Zanzibar, United Republic of Tanzania,* [2]*US President's Malaria Initiative, United States Agency for International Development, Dar es Salaam, United Republic of Tanzania,* [3]*US President's Malaria Initiative, US Centers for Disease Control and Prevention, Dar es Salaam, United Republic of Tanzania,* [4]*RTI International, Dar es Salaam, United Republic of Tanzania*

Insecticide resistance in malaria vectors has the potential to compromise the effectiveness of indoor residual spray (IRS). To ensure transmission-interrupting tools remain effective, insecticide resistance monitoring is important. Between March and October 2019, we assessed the residual efficacy of an organophosphate insecticide, pirimiphos-methyl (Actellic 300CS), sprayed on different wall surfaces inside houses. Insecticide susceptibility profiles of *Anopheles gambiae* s.l. to pyrethroids, carbamates, and organophosphates were also assessed. WHO wall cone bioassays were conducted monthly with laboratory susceptible *An. gambiae* s.s. R70 on different types of walls sprayed with pirimiphos-methyl. The wall surfaces were mud, oil- or water-painted, lime-washed, un-plastered cement block, and stone blocks. Insecticide susceptibility was tested using the standard WHO methods. Larva were collected from breeding sites in Zanzibar and reared to adults at local insectaries. The cytochrome P450 monooxygenase (P450) inhibitor piperonyl butoxide (PBO) was used to assess the role of P450s in pyrethroid resistance in *Anopheles gambiae* s.l. The residual efficacy of pirimiphos-methyl on cement, oil and water paint, lime wash, stone blocks and mud walls lasted up to 5 months with complete mortality of susceptible *An. gambiae s.s.* R70. By eight months, the average residual effect of pirimiphos-methyl remained much better on cement and both painted walls (mortality ≥75%) than other walls (mortality <75%). *Anopheles gambiae* s.l. was resistant to deltamethrin, permethrin, and alphacypermethrin (mortality <90%) but remained susceptible to bendiocarb and pirimiphos-methyl (mortality >98%). Pre-exposing *An. gambiae* s.l. to PBO restored complete susceptibility to the three pyrethroids tested indicating the involvement of P450 in observed pyrethroid resistance. While pirimiphos-methyl is still effective for IRS, after six years of use we might consider new insecticides such as clothianidin (Sumishield) and clothianidin + deltamethrin (Fudora Fusion) as part of a pro-active insecticide resistance management strategy in Zanzibar.

312

VECTOR COMPETENCE IN A SEX-BIASED TRANSGENIC STRAIN OF ANOPHELES COLUZZII MOSQUITOES

Priscila Bascunan[1], Chester J. Joyner[2], Monica Cabrera-Mora[3], Rachel Rotenberry[1], Erica Aviles[1], Eryu Wang[4], Mark Q. Benedict[1], Mary Galinski[5], Scott Weaver[4], Ellen M. Dotson[1]

[1]*DPDM/Entomology Branch, Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA 30329, Atlanta, GA, United States,* [2]*Emory Vaccine Center, Yerkes National Primate Research Center, Emory University. Current address: Center for Vaccines and Immunology, University of Georgia, Athens, Atlanta, GA, United States,* [3]*Emory Vaccine Center, Yerkes National Primate Research Center, Emory University, Atlanta, GA, United States,* [4]*University of Texas Medical Branch (UTMB), Institute for Human Infections and Immunity and Department of Microbiology & Immunology, Galveston, TX, United States,* [5]*Emory Vaccine Center, Yerkes National Primate Research Center, Emory University & Department of Medicine, Division of Infectious Diseases, Emory University School of Medicine, Atlanta, GA, United States*

Anopheles mosquitoes naturally transmit several infectious diseases such as malaria and the o'nyong-nyong virus (ONNV), taking hundreds of thousands of lives every year. Manipulating the sex ratios of wild mosquito populations could result in population suppression and thus, in a reduction in the burden of vector-borne disease in affected regions. Mali-NIH_Ac(PMB)1 is a transgenic strain of *Anopheles coluzzii*, one of the main vectors of malaria in Africa, which contains a transgene that expresses two fluorescent markers, a red fluorescent protein (DsRed) and an enhanced green fluorescent protein (eGFP) that is fused to a variant of the homing endonuclease I-PpoI (eGFP::I-PpoI124L). The expression of the endonuclease occurs during spermatogenesis and specifically cleaves ribosomal gene sequences, which are located on the X chromosome, resulting in sperm containing mostly Y chromosomes, and therefore, producing predominantly (ca. 95%) male progeny. Prior to the potential release of any transgenic mosquito strain, the vector competence must be assessed to ensure that the inserted transgenes do not have the unintended effect of increasing the mosquito's ability to transmit diseases to the human population. In this study, we examined whether the presence of the transgene in Mali-NIH_Ac(PMB)1 females affected the vector competence for *Plasmodium falciparum* NF54 strain and the ONNV in comparison to their non-transgenic siblings. The transgene of this strain had no effect on oocyst prevalence and intensity of infection for *P. falciparum*, nor on the rates of ONNV viral infection, dissemination and transmission potential.

313

EVALUATING THE ENTOMOLOGICAL EFFECTS OF ADJUNCTIVE IVERMECTIN MASS DRUG ADMINISTRATION FOR MALARIA CONTROL IN THE BIJAGOS ARCHIPELAGO, GUINEA-BISSAU: A CLUSTER-RANDOMISED TRIAL (MATAMAL)

Elizabeth Pretorius, Anna Last, James Logan, Robert Jones

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

The Global Strategic Framework for Integrated Vector Management, set out by the World Health Organization (WHO), advocates the use of a range of interventions, used collaboratively and synergistically, to help control the transmission of vector-borne diseases. Ivermectin (IVM) has the capacity to not only be integrated into existing control measures for malaria but also into existing control measures for other vector-borne diseases, leading to more cohesion between programmes. Ivermectin could work alongside indoor residual spraying and long-lasting insecticidal net (LLIN) distribution programmes in seasonal malaria transmission areas. Our trial will be the first to investigate the impact of adding IVM to a MDA with an efficacious antimalarial (Dihydroartemisinin-Piperaquine (DP)) to reduce malaria transmission in a seasonal low-transmission setting. The Bijagos archipelago is situated off the coast of Guinea-Bissau. These islands will be the site for the cluster-randomised trial of adjunctive IVM

mass drug administration (MDA). The islands will be randomly assigned to either the DP-only or DP+IVM arm (10 clusters per arm). The intervention will be implemented for two years consecutively. To investigate the effect of IVM on the mosquito population we will assess the age or parity of the mosquitoes. Collecting from indoor CDC light traps and dissecting in our field laboratory. In collaboration with the MRC the Gambia at LSHTM, we will also monitor the mosquito populations' species composition, density, susceptibility to IVM and insecticide resistance during the trial. Multivariate random effects logistic regression or generalised estimating equation modelling (adjusting for the effects of clustering) will be used to analyse the parity of the mosquito populations. Linear regression models will be used to analyse continuous outcomes (with transformation as appropriate to approximate to the normal distribution) such as mosquito density. Potential confounding factors such as LLIN coverage and use, access to health care facilities and equity bias will be included in our data collection and used to adjust the analysis.

314

INSECTICIDE RESISTANCE STATUS OF *ANOPHELES GAMBIAE* S.L. AFTER SIX YEARS OF INDOOR RESIDUAL SPRAYING IN ATACORA DEPARTMENT, NORTHERN BENIN

Rock Yves Aikpon[1], Gil Padonou[2], Fortuné Dagnon[3], Patrick Condo[1], Cyriaque Affoukou[1], Martin Akogbeto[2], Ahmed Saadani Hassani[1], Seth Irish[4], Daniel Edward Impoinvil[4], Aurore Ogouyemi Hounto[1]

[1]*NMCP Benin, Cotonou, Benin,* [2]*Centre de Recherche Entomologique de Cotonou, Cotonou, Benin,* [3]*US President's Malaria Initiative, US Agency for International Development, Cotonou, Benin,* [4]*US President's Malaria Initiative, Centers for Disease Control and Prevention, Atlanta, GA, United States*

After six consecutive years (2011 to 2016) of indoor residual spraying (IRS) in the Natitingou and Toukountouna districts of Benin, the National Malaria Control Program assessed malaria vector insecticide susceptibility to bendiocarb, a carbamate (used from 2011 to 2012) and pirimiphos-methyl, an organophosphate (used from 2013 to 2016). WHO insecticide susceptibility tests using 0.1% bendiocarb and 0.25% pirimiphos methyl were run on adult 2-5-day old F0 generation *Anopheles gambiae* s.l. This was done in both districts prior to the 2011 and 2013 IRS campaigns, and after the 2016 IRS campaign. Molecular tests were done to identify species within the *Anopheles gambiae* (s.l.) complex and the frequency of the G119S acetylcholinesterases-1 (Ace-1) mutation. Oxidase and esterase bioassays were also done to identify metabolic resistance mechanisms. Molecular tests of *An. gambiae* s.l. identified *An. gambiae* s.s. (124/147) and *An. coluzzii* (23/147). With the WHO test, the mortality rate of *An. gambiae* s.l. to bendiocarb was 97.0% in 2011 and 61.9% in 2016 in Natitingou ($p < 0.0001$) and 96.0% and 63.2% respectively in Toukountouna ($p < 0.0001$). In contrast, 100% mortality of vectors to pirimiphos methyl was observed in 2013 and 2016 for both districts. In 2011 and 2016, Ace-1 frequency respectively increased from 0.02 to 0.125 in Natitingou ($p < 0.0001$) and 0.00 to 0.133 in Toukountouna ($p < 0.0001$). Both districts also had higher oxidase and esterase activity in field collected mosquitoes compared to a susceptible strain of *An. gambiae* s.s. Kisumu. The decreased susceptibility of *An. gambiae* s.l. to bendiocarb, the increased frequency of the Ace-1 mutation, and the elevated levels of oxidase and esterase following IRS suggests that bendiocarb resistance in Benin may be multifactorial including target-site mutations and metabolic resistance. Further analysis is needed to characterize bendiocarb resistance mechanisms. While no resistance to pirimiphos methyl was observed, preserving malaria vector susceptibility to this insecticide by insecticide rotation should remain a priority for IRS in Benin.

315

LONG-LASTING INSECTICIDAL NET (LLINS) PERFORMANCE AND LONGEVITY IN VARIOUS FIELD CONDITIONS IN AFRICA, EXAMPLE OF BENIN

Idelphonse Bonaventure Ahogni[1], Jean Fortune Dagnon[2], Germain Gil Padonou[1], Patrick Condo[2], Seth Irish[3], Daniel Edward Impoinvil[3], Ahmed Saadani Hassani[2], John E. Gimnig[3], Martin Codjo Akogbeto[1]

[1]*Centre de Recherche Entomologique de Cotonou, Cotonou, Benin,* [2]*US President's Malaria Initiative, US Agency for International Development, Cotonou, Benin,* [3]*US President's Malaria Initiative, Centers for Disease Control and Prevention, Atlanta, GA, United States*

Long-lasting insecticide-treated mosquito nets (LLINs) are an essential tool for malaria control. Physical integrity, durability and bio-efficacy are key effectiveness variables for LLINs. The objective of this study was to identify the primary factors that impact the survival of seven brands of LLINs with different physical characteristics. A cohort consisting of 270 nets of each brand was surveyed every 6 months from August 2017 to September 2019 in Zagnanado District, Benin. Brands included OlysetNet®, PermaNet® 2.0, Royal Sentry®, PermaNet® 3.0 (with a reinforced border), and 3 "aspirational" nets with alternate specifications: DawaPlus 2.0 (polyester, 150denier, 40g/m^2 fabric weight), Yorkool® (polyester, 75denier, alternate knitting pattern with 85 g/m^2 fabric weight), and DCT aspirational net (polyester, 150denier, 66g/m^2 fabric weight). Overall, 644 LLINs of the 1,890 distributed were found after 24 months of use. The overall attrition rate of the LLINs was 65.9% at 24 months. The main reasons for loss were movement of nets (58.7%), accidental tears (33.2%) and repurposing (8.1%) ($p<0.001$). There was no significant difference in attrition between the different brands of LLINs ($p=0.25$). The proportionate hole index (pHI) ranged from 24 to 197 with a significantly lower pHI for the DCT aspirational net compared to the OlysetNet ($P<0.05$). After 24 months of use, 83.9% were in good condition ($0\leq pHI<65$), 12.9% were damaged ($65\leq pHI<643$) and 3.2% were too torn ($643\leq pHI$). A significant decrease in the physical survivorship of LLINs (all brands) was observed at 24 months (45.3%, range 42.7-47.9%) compared to 6 months (91.8%, range 90.5-92.9%) ($p<0.001$). The fall in the survivorship of LLINs during this study underlines the need to develop and implement new strategies for managing this important tool of vector control.

316

HUMAN BEHAVIOR AS A DETERMINANT OF MALARIA RISK IN BANDARBAN, BANGLADESH

Matthew A. Aubourg[1], Allison L. Hendershot[1], Mohammad Shafiul Alam[2], Hasan Mohammad Al-Amin[3], Neil F. Lobo[1]

[1]*University of Notre Dame, Notre Dame, IN, United States,* [2]*International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh,* [3]*QIMR Berghofer Medical Research Institute, Brisbane, Australia*

Intervention efforts over the past decade have resulted in malaria being close to elimination in Bangladesh. The use of insecticide-impregnated nets (ITNs) have reduced malaria incidence and mortality by over 75%. However, high levels of transmission persist in thirteen border belt districts, especially in the Chittagong Hill Tract districts (Bandarban, Khagrachhari, and Rangamati). High *Anopheles* vector diversity combined with deforestation, possible changing mosquito behaviors, and recent documentations of insecticide resistance likely perpetuate transmission in these areas. However, recent increased malaria incidence in these regions demonstrates that factors and drivers of malaria risk remain uncharacterized. In this study, we show how human behavior and spatiotemporal use of ITNs - the local primary intervention tool - adjust predictions of malaria exposure. While vector studies usually determine indoor and outdoor biting rates, this study characterizes human exposure - evaluating risk based on both vector and human behaviors. Preliminary analysis reveals malaria exposure outside LLIN use. Insecticide resistance observed in the important vector *An. vagus* in this region points to the

urgency in understanding local gaps in protection. Incorporating human behavior in exposure estimates demonstrates gaps in protection and may impact intervention strategies, saving time, resources, and reducing malaria. Human behavior-adjustments may be used for understanding local efficacy and limitations of vector control strategies.

317

LARVAL SOURCE MANAGEMENT IN FISH PONDS IN THE BRAZILIAN AMAZON: IMPACT OF BIOLARVICIDE APPLICATION ON *ANOPHELES DARLING* LARVAL DENSITY AND ON MALARIA TRANSMISSION

Pablo Secato Fontoura[1], Amanda Oliveira Sampaio[2], Simone Ladeia Andrade[3], Marcia Caldas de Castro[4], Marcelo Urbano Ferreira[1]

[1]*Institute of Biomedical Sciences /University of São Paulo, Sao Paulo, Brazil,* [2]*Multidisciplinary Center / Federal University of Acre, Cruzeiro do Sul, Brazil,* [3]*Laboratory of Parasitic Diseases / Oswaldo Cruz Foundation, Rio de Janeiro, Brazil,* [4]*Department of Global Health and Population / Harvard T.H. Chan School of Public Health, Boston, MA, United States*

Fish ponds opened for commercial aquaculture in the Brazilian Amazon are the dominant *Anopheles* mosquito breeding habitats near or within urban centers, favoring malaria transmission. In addition, *An. darlingi*, the most prevalent and important vector of malaria in the Amazon, has gradually changed its biting and resting behavior over the past decades, and currently typically feed and rest outdoors. Between September 2017 to October 2019 we conducted a large-scale larval control intervention in 170 fish ponds in Assis Brasil, a peri-urban area in the western Amazon (in the main foci of malaria transmission in Brazil). The main aim was to test whether the periodic application of an environmentally safe biolarvicide (VectoMax FG, 20 kg/ha) in fish farming ponds is effective to reduce larval density and decrease malaria transmission in a high-endemic area in the Brazilian Amazon. To this end, we compared entomological data and malaria prevalence and incidence estimates obtained over two years (12 months before the larviciding intervention and 12 months during the intervention). During the pre-intervention entomological monitoring (September 2017 to Ocotber 2018), we found anopheline larvae (regardless of the stage) in 1,681 (86.6%) larval habitat samplings. Ambiental variables (precipitation), characteristics of fish ponds (natural, human-made, structured margins, presence of vegetation, shaded margins), and presence of pupae and *Culex* larvae were positively correlated (p-value < 0.05; linear regression models) with larval densities in different stages. The application of larvicide started in November 2018 and ended in October 2019. To measure the impact of LSM on malaria transmission, three population malaria prevalence surveys were carried out in the study area (about 1,600 inhabitants). Based on results, the prevalence of malaria infection was 2.72% before the intervention, 0.73% during the intervention, and 0.24% at the end of the intervention. We are conducting PCR analysis of the samples. Larval, epidemiological, and local ecology data will be combined to assess the effect of the intervention on malaria transmission.

318

NON-TYPHOIDAL *SALMONELLA* FROM PAEDIATRIC STOOLS IN NORTHERN IBADAN, NIGERIA

Olabisi Comfort Akinlabi[1], Stella Ekpo[1], Ayorinde Afolayan[1], Adeola Omotuyi[1], Akinlolu Adepoju[2], Gordon Dougan[3], Iruka N. Okeke[1]

[1]*University of Ibadan, Ibadan, Nigeria,* [2]*Department of Paediatrics, College of Medicine, University of Ibadan, Oyo, Nigeria,, Ibadan, Nigeria,* [3]*Wellcome Trust Sanger Institute, Hinxton, Cambridgeshire, United Kingdom, Hinxton, United Kingdom*

Diarrhoeal diseases constitute a major public health problem, particularly in developing countries such as Nigeria, where they are the second leading cause of death in children under five. We hypothesized that non-typhoidal *Salmonella enterica* (NTS) are major aetiologic agents of infantile diarrhoea in northern Ibadan, Nigeria. Between November 2015 and August 2019, 477 stools were collected from 120 diarrheic children and 357 healthy controls attending five primary health care centers in Ibadan North metropolis. NTS isolates were confirmed biochemically, using Microbact® and PCR. Six isolates were whole genome sequenced using Illumina platform, quality control (FASTQC and Trimmomatic), genome assembly (spAdes) and speciation (BactinspectorMax). The genomes were then placed within the context of 30 globally disseminated NTS clones via single nucleotide polymorphism analysis. Nine NTS were isolated from the stool samples, four (3.3%) from children with diarrhoea and five (1.4%) from healthy children ($p>0.05$). Seven out of the nine children from whose stool *Salmonella* was isolated, including five exclusively breast-fed babies, lived in homes with wells as source of household water, prompting us to screen fifteen domestic wells within the study area. All the wells had unacceptably high coliform counts and two had NTS isolated from it. Faecal specimens yielded *Salmonella* Weltervreden (1) and *S.* Heidelberg (1) from diarrheic children while *S.* Typhimurium (1) and *S.* Agona (2) were isolated from control children. The S. Weltervreden isolate did not cluster with either of the two globally-disseminated clones but the other isolates were related to predominant isolates from elsewhere on the globe. This study recovered non-typhoidal *Salmonella* from the stool of young children in Ibadan and pointed to household use of well water as a possible risk factor for NTS spread. NTS circulating in Ibadan include globally disseminated and unique isolates. High recovery rates of NTS from healthy children suggest that there may be a role for protective factors such as breastfeeding or maternal antibodies in childhood enteric salmonellosis.

319

A COMPARISON OF TRADITIONAL DIARRHOEA SURVEILLANCE METHODS WITH STOOL MICROBIOLOGICAL INDICATORS IN THE FORCIBLY DISPLACED MYANMAR NATIONALS CAMPS IN COX'S BAZAR, BANGLADESH

Ryan T. Rego[1], Samuel I. Watson[1], Mohammad Atique Ul Alam[2], Syed Asif Abdullah[2], Mohammad Yunus[2], Mohammad Sirajul Islam[2], A.S.G Faruque[2], Azharul Islam Khan[2], John Clemens[2], Richard J. Lilford[3]

[1]*University of Warwick, Coventry, United Kingdom,* [2]*International Center for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh,* [3]*University of Birmingham, Birmingham, United Kingdom*

Water, sanitation, and hygiene (WASH) interventions aim to limit the spread of enteric pathogens, a major cause of diarrhoea. However, evidence on the efficacy of various WASH systems is mixed. This may be due to the use of non-objective and reactive methodologies in the measurement of population-level enteric infection, commonly door-to-door questioning of carers for diarrhoea - the main clinical presenting symptom of enteric infection. The relationship between diarrhoea and infection though is uncertain, given non-pathogenic diarrhoea and asymptomatic infection. We conducted a study among children under five to compare parental report of diarrhoea (using both pictorial and verbal questioning) against visual and microbiological stool analysis. A subset of children was also examined for malnutrition. The study took place at two time points to account for seasonality. Overall, 124 stool samples were collected and tested from 714 surveyed households. To date, all surveys have taken place, as well as PCR analyses for proteins and viruses (with bacteriology and parasitology forthcoming summer 2020). The overall reported diarrhoea rate was 74% in the pictorial survey and 31% in the verbal. 58% of stools were visually identified as loose or watery, and 57% contained any enteric virus (72% in the dry season and 40% in the rainy season). The sensitivity of diarrhoea for identifying viral infection was 59% for the pictorial survey and 45% for the verbal survey, and 54% for visual analysis. 76% of stools had elevated protein levels, with 33.9% having levels associated with irritable bowel syndrome. The differences between diarrhoea report methods and stool visual analysis suggest bias in parental diarrhoea report. Protein results indicate that a large proportion of children have chronic diarrhoea and infection. The low sensitivity of diarrhoea as an indicator of viral infection suggests a poor relationship between diarrhoea

and infection. Forthcoming bacteriology and parasitology results will allow for examination of the sensitivity of diarrhoea for all types of infection, and estimation of the specificity of diarrhoea as a marker of infection.

320

MULTIPLE ANTIBIOTIC RESISTANCE IN *ESCHERICHIA COLI* ISOLATED FROM STOOL SAMPLES OF HEALTHY INFANTS IN RURAL BANGLADESH

Fatema-Tuz Johura[1], Jarin Tasnim[1], Sahitya Ranjan Biswas[1], Riajul Islam[1], Talal Hossain[1], Kazi Sumaita Nahar[1], Rehnuma Binte Kabir[1], Indrajit Barmon[1], Susmita Basak[1], Farzana Hossain[1], Wali Ullah[1], Marzia Sultana[1], Hafizur Rahman[2], Saijuddin Shaikh[2], Hasmot Ali[2], Subhra Chakraborty[3], Alain Labrique[3], Tahmeed Ahmed[1], Md. Iqbal Hossain[1], Munirul Alam[1], Amanda C. Palmer[3]

[1]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh,* [2]*The JiVitA Project, Gaibandha, Bangladesh,* [3]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States*

Global concern over antibiotic resistance is escalating, as health professionals face increasing challenges in tackling severe infectious diseases. *Escherichia coli* is a commensal in humans and animals. It plays an important role in growth and development, particularly in the immune system. The bacterium also serves as a reservoir for antibiotic resistance, thus providing an indicator of drug resistance patterns in a community. We investigated antibiotic resistance in *E. coli* isolated from non-diarrheal stool samples of 6-mo-old infants (n=128) from Gaibandha, in northwest Bangladesh. We conducted susceptibility testing on the isolates by disc diffusion assay against 20 antibiotics. Resistance profiles were assigned following CLSI cut-offs. A random 38% subset was selected to test for minimum inhibitory concentration (MIC) of azithromycin using E-test. Of the 128 stool samples tested, 110 (86%) yielded *E. coli* using standard culturing methods. *E. coli* carried resistance markers for 14 important antibiotics, including erythromycin (98.2%), azithromycin (64.5%), ampicillin (70.0%), amoxicillin/clavulanate (12.7%), ceftriaxone (33.6%), cefuroxime (33.6%), cefepime (20.9%), ceftazidime (10.0%), ciprofloxacin (33.6%), levofloxacin (18.2%), tetracycline (30.9%), doxycycline (13.6%), trimethoprim/sulfamethoxazole (33.6%), and aztreonam (21.8%). Fosfomycin, gentamicin, and meropenem were the most effective antibiotics with 100% sensitivity, followed by mecillinam (99.1%), tigecycline (98.2%) and imipenem (98.2%). All isolates except one were resistant to at least one antibiotic and 57.3% were multidrug-resistant (resistant to at least one agent in ≥3 antimicrobial categories). The MIC for azithromycin ranged from 32-256µg/ml, with a median of 256 µg/ml (25^{th}, 75^{th} percentiles: 56 µg/ml, 256 µg/ml). Our results likely reflect the environmental burden of resistant *E. coli* and prevailing drug resistance patterns in this community. They also point to the human gut as an important reservoir for multiple antibiotic resistances, which could potentially jeopardize therapeutic intervention.

321

DETECTION OF ENTERIC PATHOGENS AND CONTINUATION OF DIARRHEA AMONG CHILDREN WITH MODERATE-TO-SEVERE DIARRHEA ENROLLED IN THE VACCINE IMPACT ON DIARRHEA IN AFRICA (VIDA) STUDY: KENYA, 2015-2018

Graeme Prentice-Mott[1], David M. Berendes[1], Perrine Marcenac[1], Talia Pindyck[1], Sunkyung Kim[1], Richard Omore[2], Ciara E. O'Reilly[1], John B. Ochieng[2], Jane Juma[2], Jennifer R. Verani[3], Marc-Alain Widdowson[4], Yuanyuan Liang[5], Helen Powell[6], Karen L. Kotloff[7], Eric D. Mintz[1]

[1]*Division of Foodborne, Waterborne, and Environmental Diseases, Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Kenya Medical Research Institute, Center for Global Health Research, Kisumu, Kenya,* [3]*Division of Global Health Protection, Centers for Disease Control and Prevention, Nairobi, Kenya,* [4]*Institute of Tropical Medicine Antwerp, Brussels, Belgium,* [5]*Department of Epidemiology and Public Health, University of Maryland School of Medicine, Baltimore, MD, United States,* [6]*Department of Pediatrics, Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States,* [7]*Department of Medicine, Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States*

Among infants and young children in low- and middle-income countries, persistent diarrhea (≥14 days) is associated with stunting, malnutrition, decreased cognitive function, and mortality. We assessed the association of detection of enteric pathogens with continuation of diarrhea in children <5 years old with moderate-to-severe diarrhea (MSD) enrolled in the Vaccine Impact on Diarrhea in Africa (VIDA) study in Kenya from 2015-2018. We used Cox regression to estimate hazard ratios (HR) for the time to end of a diarrhea episode (2 days with no diarrhea) from post-enrollment diarrhea days recorded on memory aid forms. Associations were estimated for presence/absence and cycle threshold (Ct) value of pathogen in stool at enrollment from TaqMan Array Cards (TAC), which were modelled quantitatively using penalized splines. Models were adjusted for age, nutritional status, household wealth, home treatment with oral rehydration salts or zinc, antibiotic treatment, and duration of pre-enrollment diarrhea. Of 1,482 MSD cases, 1,004 (68%) had acute diarrhea (1-6 consecutive days), 420 (28%) had prolonged acute diarrhea (7-13 days), and 58 (4%) had persistent diarrhea. At a given day in the course of a diarrheal episode, the probability of the episode resolving was lower in children with detection in stool of *Shigella* spp. (HR: 0.83, 95% confidence interval (CI): 0.74-0.94) and typical enteropathogenic *E. coli* (HR: 0.77, 95% CI: 0.67-0.90). In quantitative assessments, the probability of diarrhea resolving was reduced at higher pathogen concentrations (lower Ct value) for *Campylobacter coli* or *jejuni* and, notably, *Shigella* spp. (HR *Shigella* spp. at Ct=30: 0.80, 95% CI: 0.72-0.89; HR Ct=25: 0.65, 95% CI: 0.53-0.80; HR Ct=20: 0.55, 95% CI: 0.41-0.73, respectively). We identified pathogens associated with continuation of a diarrheal episode and some evidence of dose-response relationships with specific bacterial pathogens, which may indicate mechanisms involved in persistence of diarrhea. These findings highlight the importance of preventive and therapeutic measures for pathogens associated with prolonged diarrheal illness.

322

PREDICTORS OF MULTI-DRUG RESISTANCE OF ENTERIC PATHOGENS IN PATIENTS WITH ACUTE DIARRHEA IN BANGLADESH

Stephanie C. Garbern[1], Sabiha Nasrin[2], Monique Gainey[3], Nur H. Alam[2], Adam C. Levine[1]

[1]*Brown University, Providence, RI, United States,* [2]*icddr,b, Dhaka, Bangladesh,* [3]*Rhode Island Hospital, Providence, RI, United States*

Antimicrobial resistance (AMR) is a global public health threat and is increasingly prevalent among enteric pathogens in low- and middle-income countries (LMICs). However, the burden of multi-drug resistance (MDR) in patients with acute diarrhea in LMICs is poorly understood. This study's aim was to characterize the prevalence of MDR among enteric pathogens isolated from patients with acute diarrhea presenting for emergency care in Bangladesh and assess clinical and sociodemographic factors associated with MDR. This study was a secondary analysis of data collected March 2019 - January 2020 from adults and children over five years old with acute gastroenteritis at icddr,b Dhaka Hospital in Bangladesh. A wide range of clinical data including a stool sample for culture and antimicrobial susceptibility testing was collected. Logistic regression was used to assess the association between clinical predictors and presence of MDR pathogens (resistance to an antibiotic in >3 antibiotic classes). A total of 1,078 patients had growth present on stool culture with antimicrobial susceptibility results with overall prevalence of MDR 26.2%. MDR prevalence by pathogen was: *Vibrio cholera* (13.6%), *Aeromonas* spp (79.1%), *Campylobacter* spp (76.63%), *Salmonella* spp (5.7%) and *Shigella* spp (24.3%). Factors associated with increased odds of having MDR included age < 18 years (OR 1.49, 95%CI 1.18-1.88)), having more than 10 episodes of diarrhea (OR 1.30, 95%CI 1.03-1.62),

decreased radial pulse (OR1.54, 95%CI 1.25-1.89), decreased urine output (OR 1.33 95%CI 1.06-1.66). Having a flush toilet (OR 0.81, 95%CI 0.66-0.99) was associated with decreased odds of MDR. MDR in enteric pathogens was widespread in this study population. Younger age, number of diarrheal episodes, signs of significant dehydration and poor sanitation were correlated with MDR enteric pathogen infections among patients in Bangladesh. These findings may help guide clinical decision-making in patients at greatest risk of complications due to MDR.

323

BACTERIAL ISOLATION AND SUSCEPTIBILITY PATTERN OF DIARRHEAL PATHOGEN IN OLDER CHILDREN AND ADULTS IN DHAKA HOSPITAL, BANGLADESH

Sabiha Nasrin[1], Stephanie C. Garbern[2], Monique Gainey[3], Nur H. Alam[1], Adam C. Levine[2]

[1]*icddr,b, Dhaka, Bangladesh,* [2]*Brown University, Providence, RI, United States,* [3]*Rhode Island Hospital, Providence, RI, United States*

Antibiotic Resistance (ABR) is a global problem driven by poor healthcare infrastructure and misuse or overuse of antibiotics among other factors. Bangladesh presents a regional and global threat with a high degree of ABR. Thus, up to date information is required on local etiology and antimicrobial susceptibility pattern to aid clinicians in select appropriate antibiotics. We aimed to identify the antibiotic susceptibility pattern of bacteria causing diarrhea, to better treat these patients with appropriate antibiotic. Data collected from patients over five years of age with acute diarrhea presenting to Dhaka hospital from March to January 2020. Stool samples were collected from each patient and sent for culture sensitivity testing by disc diffusion method. A total of 1945 patients were enrolled with 1898 patients stool cultures available for further analysis. A total of 1078 (56.83%) patients had positive culture of bacterial etiology of diarrhea. Among them *V. Cholerae* was isolated from 470 (43.60%), *Shigella* from 22 (2.04%), *Campylobacter* 65 (6.03%), *Salmonella* 34 (3.15%), *Aeromonas* 329 (30.52%) and other organism 11 (1.0%) patients. Azithromycin (97% sensitive), Ciprofloxacin (98%) and Tetracycline (98%) were found to be more effective antibiotic against *V. Cholerae.* Ceftriaxone (90%), Mecillinam (78%) and Azithromycin (70%) were most effective for *Shigella*. For *Campylobacter,* highest sensitivity was seen to Gentamycin (94%) followed by Tetracycline (84%). All *Salmonella* isolates were highly sensitive against most commonly used antibiotics. On the contrary *Aeromonas* pathogen found to be resistant for most of the antibiotics. Among them, highest sensitivity was found for Erythromycin (94%). Azithromycin was found to be an effective antibiotic against all pathogens except *Aeromonas* in this study. However, each pathogen was resistant to at least one antibiotic. Highest resistance was found for *Aeromonas*. Continuous research is needed to know antibiotic susceptibility pattern to use suitable antibiotic timely.

324

CLINICAL PREDICTORS OF ACUTE DIARRHEAL DISEASE WITH BACTERIAL ETIOLOGY IN ADULTS AND OLDER CHILDREN IN BANGLADESH

Monique Gainey[1], Stephanie C. Garbern[2], Adam C. Levine[2], Sabiha Nasrin[3], Nur H. Alam[3]

[1]*Rhode Island Hospital, Providence, RI, United States,* [2]*Brown University, Providence, RI, United States,* [3]*icddr,b, Dhaka, Bangladesh*

Diarrheal disease is the 5th leading cause of years of life lost globally accounting for over 1.3 million deaths in 2015. However, clinical predictors of bacterial diarrhea among adults and children over 5 have not been fully investigated. This study's aim is to investigate the clinical predictors for bacterial etiology in acute gastroenteritis among adults and children over five years old. This study was a secondary analysis of data collected from March 2019-Jauary 2020 of adults and children over five years old presenting with acute gastroenteritis at icddr,b's Dhaka Hospital in Bangladesh. A wide range of clinical/historical data was collected including a stool sample for culture and PCR testing. Pearson's chi-square analysis and logistic regression were used to assess the association between clinical predictors and stool testing indicating bacterial etiology. A total of 1,897 patients had a completed stool culture/PCR result for inclusion in the secondary analysis with 1,371 (72.3%) testing positive for bacterial diarrhea. Significant clinical predictors of bacterial illness included: sex (X^2=5.21, p=0.022), sunken eyes (X^2=6.43, p=0.011), weak radial pulse (X^2=27.54, p<0.001), deep respirations (X^2=6.30, p=0.012), decreased urine output (X^2=6.18, p=0.046), rice water stool color observed by study staff (X^2=24.85, p<0.001) and prior use of antibiotics for the current illness (X^2=14.41, p<0.001). The odds of having bacterial diarrhea decreased with higher systolic (OR=0.99, 95% CI 0.98-0.99), diastolic blood pressure (OR=0.98, 95% CI 0.98-0.99) and temperature (OR=0.91, 95%CI 0.84-0.98) and increased with higher heart rate (OR=1.01, 95% CI 1.00-1.01). Heart rate, temperature, blood pressure, weak radial pulse, deep respirations, decreased urine output, sunken eyes and stool color were predictive clinical signs of a positive stool culture/PCR in this study population. These findings may help guide further research to develop predictive tools to aid clinicians in identifying patients with bacterial diarrhea to allow for timely and appropriate use of antibiotics.

325

ACUTE NOROVIRUS GASTROENTERITIS AMONG INTERNATIONAL TRAVELERS: RESULTS FROM A PROSPECTIVE COHORT STUDY

Robert Steffen[1], Christine L. Moe[2], Christoph Hatz[3], Martin Alberer[4], Hans D. Nothdurft[4], Lisa Lindsay[5], Amy E. Kirby[2], Henry M. Wu[6], Thomas Verstraeten[5], Herbert L. Dupont[7]

[1]*University of Zurich, Epidemiology, Biostatistics and Prevention Institute, WHO Collaborating Centre for Travellers' Health, Zurich, Switzerland,* [2]*Emory University, Rollins School of Public Health, Atlanta, GA, United States,* [3]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [4]*Division of Infectious Diseases and Tropical Medicine, University Hospital, Ludwig-Maximilians-University (LMU) Munich, Munich, Germany,* [5]*P95 Epidemiology and Pharmacovigilance, Leuven, Belgium,* [6]*Emory University Travel Well Clinic, Atlanta, GA, United States,* [7]*University of Texas McGovern Medical School of Public Health, Houston, TX, United States*

Noroviruses (NoV) are the most frequent cause of acute gastroenteritis (AGE) outbreaks worldwide, and a well-known cause of AGE outbreaks on cruise ships. We aimed to estimate the burden of NoV-related AGE acquired during international travel in adults.This was a multi-center prospective cohort study. Volunteers ≥ 18 years were recruited in travel clinics in Europe and the USA if they were to travel internationally for 3-14 days to either low- or middle-income countries (LMIC), or on a cruise that included an international port stop.AGE symptoms were recorded in a diary. Travel-acquired AGE was defined as the presence between day 2 of travel and day 2 post-travel of: 1) any vomiting; or 2) three or more loose stools within 24 hours; or 3) two loose stools plus another symptom within 24 hours. Post-travel stool samples were collected from all symptomatic travelers within 2 weeks of returning and tested for NoV via qRT-PCR. If positive, acquisition during travel was confirmed with the pre-travel stool sample. A random subset of symptomatic post-travel stool samples were tested for other common enteric pathogens via xTAG®GPP Luminex. Travelers were included in the analysis if they provided a pre-travel stool sample and completed one diary entry.Overall, 1386 travelers were enrolled, of whom 1092 were included in the analysis. Travel occurred between March 2015 and June 2017, with Latin America (51%) and Asia (24 %) as the most frequent destinations. A total of 395 out of 1092 (36.2%) travelers developed travel-acquired AGE. Pre- and post-travel stool samples were available for testing for 308. NoV infections were detected in 20/308 (6.5%). In addition, 25 (13.3%) of the 188 travelers that were selected for multiplex assay testing were positive for any pathogen, of which ETEC was the most frequent (19/188, 10.1%).We found that approximately 1 in 50 travelers to LMIC experience NoV-related AGE. The incidence of NoV and other pathogens may have been underestimated because some post-travel stool samples were collected relatively late after symptoms, as illustrated by the low proportion of AGE cases in which a pathogen was identified.

326

ANTIMICROBIAL RESISTANCE IN KENYA AND EFFORTS TOWARDS ADDRESSING THE CHALLENGE

Samuel M. Kariuki[1], Cecilia K. Mbae[1], Susan K. Kavai[1], Evelyne Wesangula[2], Robert Onsare[1]

[1]*Kenya Medical Research Institute, 43640-Nairobi, Kenya,* [2]*Ministry of Health, Nairobi, Kenya*

In Kenya, Antimicrobial Resistance (AMR) in enteric pathogens is a major problem especially in disease endemic areas including informal settlements where WaSH infrastructure is poor and access to antimicrobials without prescription is common. In the last 10 years we have experienced challenges in treatment of major diseases of public health significance as alternative treatment options are either too expensive or totally unavailable for these vulnerable populations. In this period, we studied the epidemiology and genomics of Multidrug-resistant (MDR) enteric pathogens; Salmonella, *E. coli* and *Vibrio cholerae*, underscoring the importance of implementing the National Action Plan (NAP) for prevention and containment of AMR in a truly One-Health approach. We present AMR data on invasive non-typhoidal Salmonella (NTS) among children (0-10 years) and revealing an increasing burden of typhoid in this age group. 67% of iNTS isolates are MDR, with 15-20% of isolates showing reduced susceptibility to fluoroquinolones and producing extended-spectrum-beta-lactamases (ESBLs). We also present data on cholera outbreaks indicating transmission of MDR strains from new hotspots in refugee camps and informal settlements around the city of Nairobi. At least 55% of all our *V. cholerae* isolates are now resistant to 3 or more commonly available drugs. From 2012 among *V. cholerae* and NTS, ESBL-producing strains (CTX-M-15) have emerged; these now form 8-15% of isolates among outpatients in the informal settlements. In conclusion, among the populations of enteric pathogens, there is unprecedented exchange of mobile genetic elements (MGEs) within disease endemic hotspots. The acceleration of implementation of NAPs to combat AMR will be crucial to contain AMR in these settings. Vaccination strategies, in absence of immediate solutions to poor WaSH infrastructure, will be a viable option for control and prevention of these diseases.

327

MOLECULAR CHARACTERIZATION OF EXTENDED SPECTRUM BETA LACTAMASE (ESBL) PRODUCING *ESCHERICHIA COLI* AMONG WOMEN IN ANAMBRA STATE, NIGERIA

Uchenna C. Ogwaluonye[1], Ugochukwu M. Okezie[1], Hopewell Nnaji[1], Chukwuebuka C. Ezeagu[2], Malachy C. Ugwu[1], Edward Nwanegbo[1]

[1]*Nnamdi Azikiwe University, Awka, Nigeria,* [2]*African Health and Sustainability Mission (N.G.O), Enugu, Nigeria*

Maternal death from sepsis is a major problem in Nigeria; the Nation's lifetime risk of maternal death is 1 in 13 according to African Populations and Health Research Centre. Genital and urinary tract infections are major risk factors and *Escherichia coli* is a major causative organism. This pilot study was designed to molecularly screen *Escherichia coli* for presence of ESBL resistance genes, among 56 asymptomatic pregnant and non-pregnant women (aged 15-49 years) at Chukwuemeka Odumegwu Ojukwu Teaching Hospital, Anambra State, Nigeria. *Escherichia coli* isolates were tested against a panel of antibiotics and screened for Extended Spectrum Beta Lactamase (ESBL) using the double disc synergy test (DDST). This was followed by molecular characterization of the isolates to test for presence of Bla_{TEM}, Bla_{SHV}, Bla_{CTX-M}, Bla_{CMY-2} (pAmpC), Bla_{IMP} (MBL gene) and Bla_{VIM}(MBL gene) using PCR and agarose gel electrophoresis. Overall, an incidence of 10.7% was found among the study subjects. The incidence was higher in pregnant (33.3%) than in non-pregnant (2.4%) women. Isolates demonstrated different antibiotic resistance levels as follows: amoxicillin/clavulanate (100%), Ceftazidime (50%), Co-trimoxazole (50%), Ceftriaxone (33.3%) and Ofloxacin (33.3%). No resistance was recorded for Gentamicin, Ciprofloxacin and Nitrofurantoin. Four isolates exhibited Bla_{CTX-M} gene while only one isolate demonstrated the Bla_{TEM} gene. Widespread ESBL resistance genes (CTX-M & TEM) identified in this study calls for an in-depth review of antibiotic treatment policies of Nigeria healthcare systems, especially with regards to the routine use of cephalosporins and penicillins for treatment of suspected Urinary tract infection among women. The relevance of molecular characterization in determining the presence of resistance genes in urinary bacteria isolates warrants further evaluation and implementation in health institutions especially in LMICs like Nigeria.

328

SPECIES IDENTIFICATION OF MEALIE MEAL SPOILAGE ORGANISMS AND PATHOGENIC BACTERIA FROM SELECTED FOOD STORES IN LUSAKA DISTRICT OF ZAMBIA

Dayo Omodele Adeyemo, Bernard Hang'ombe, John Muma, Choolwe Munkombwe, Muso Munyeme, Kaunda Ndashe

University of Zambia, Lusaka, Zambia

Mealie meal constitutes greater part of the daily diet for growing population of Lusaka District in Zambia. However reported cases of cholera outbreak in October 2017-May 2018 were attributed to water contamination in the households of Lusaka. Hitherto there is no previous study or documented data for gastroenteritis caused by microbial contamination from food sources. Hence this study was carried out from January - March 2020 for species identification of maize meal flour spoilage organisms and pathogenic bacteria from selected food stores in Lusaka district. Thus, Lusaka district was mapped out into five study areas namely: South, Central, East, West and North. Food stores in each area were further stratified into sampling unit of interest: mall, shop and street food vendor. Cross sectional survey was conducted for food spoilage organisms and pathogenic bacteria of packaged maize meal flour from selected food stores in each sampling unit. To estimate the prevalence within 5% allowable error and considering a 95% confidence level, the calculation will be based on the assumption that 50% of the maize flour samples collected from Food stores in Lusaka district have microbial contamination. Therefore the packaged maize meal flour samples from selected Food Stores included in the study was 158 samples. Subsequently isolation by spread plate method and species identification of microbial contamination by Cell Culture processes coupled to the microbial morphological, physiological and biochemical characterization were performed. The preliminary laboratory findings revealed the followings: Spoilage organisms - *Aspergillius* species, *Mucor* Species , *Rhizopus* species , *Candida* species , *Clostridium* species and *Bacillus* species; Pathogenic Bacteria species - *Clostridium perfringens, Staphlococus aureus, Clostridium tetani , Bacillus, Escherichia coli, Pseudomonas aeruginosa and Yersinia pestis*. In conclusion, it can be established from the aforementioned laboratory results that food poisoning outbreak could evolve from food stores in Lusaka district apart from water contamination.

330

SERO PREVALENCE AND MOLECULAR EPIDEMIOLOGY STUDY OF BRUCELLOSIS IN EASTERN ETHIOPIA

Getachew Abichu Duressa

National Animal Health Diagnostic and Investigation Center, Addis Ababa, Ethiopia

The main objective of this study is to determine the seroprevalence and identification of the causative agent, in Cattle, Small ruminant and camel from Eastern part of Ethiopia. A total of 698 blood samples were collected from cattle, Sheep goats and camel. Data related to age, sex, location, and breed were collected on the sampling day. Serum samples was initially screened using C-ELISA. Seropositive samples were subjected to bacterial PCR analysis using Brucella species-specific (IS711 for *Brucella abortus* and *B. melitensis*) real-time polymerase chain reactions (RT-PCR Forty three (6.1%) serum samples were positive C-ELISA. Of the 43 seropositive serum samples, 20 (46.5%) were positive in the *B. abortus*-specific (IS711) RT-PCR. Of which 19(95%) were *B. melitensis* from all samples, only 1(5%) was *B. abortus* (5%). In conclusion: *B. melitensis* and *B. abortus*

were identified as causative agent of Camel, *B. melitensis* was for the causative agent of ovine and caprine brucellosis in the study area. Based on the study result screening of brucellosis should be practiced before an introduction of animal for breeding purpose and used as consumption of milk for human.

332

BACTERIOPHAGE DISCOVERY AND SPECIFICITY AGAINST CLINICALLY RELEVANT *STAPHYLOCOCCUS AUREUS* ISOLATES FROM WOUND INFECTIONS IN THE PERUVIAN AMAZON RIVER BASIN

Abby London[1], Christopher Messner[1], Ricardo Abadie[2], Andrew Nevin[1], Amanda Williams[1], David Craft[1], James Regeimbal[2]

[1]*Penn State College of Medicine, Hershey, PA, United States,* [2]*Naval Medical Research Unit – Six, Lima, Peru*

Investigating novel therapies, such as lytic bacteriophage (phage), has become an important factor in addressing the global concern of emerging antibiotic resistance. Of interest is developing specific therapy to treat community and nosocomial acquired *Staphylococcus aureus* infections. The purpose of this study was to harvest and investigate specificities of phage against clinically relevant *S. aureus* isolates from wound infections. Previously collected swabs from wound infections yielding methicillin resistant *S. aureus* (MRSA), methicillin sensitive *S. aureus*, and coagulase-negative staphylococci from hospitals in Iquitos, Peru were cultured in tryptic soy broth and incubated overnight with shaking at 37°C. The culture was centrifuged and supernatant filtered for phage isolation. Serial dilutions of the phage were spotted on plates against 20 different clinical MRSA isolates from wound infections in a similar community and incubated overnight at 37°C. Plaques demonstrating lytic activity were picked and placed in PBS for 1 hour at room temperature. The samples were vortexed and filtered to obtain phage and stored at 4°C. These phages were also plated against strains of *S. aureus* previously cultured in the presence of *S. aureus* specific phage. Phage isolates showed efficacy against 9/20 clinical isolates of MRSA. None of the phage demonstrated lytic activity against phage resistant *S. aureus.* Our hypothesis that we would isolate phage from wound infections with specificity against 20 MRSA strains from wound infections in a similar community was proven. However, our hypothesis that this phage would be efficacious against previously characterized phage resistant *S. aureus* was not proven. This may suggest there is a selection occurring between the bacteria and the phage that allows the bacteria to adapt to the phage. The adaptation may be related to specificity and cell wall modifications, or a genotypic change related to lytic and lysogenic activity. Our results demonstrate the need for large phage libraries and further characterization of multiple phages with varying specificities for therapeutic response.

333

EVALUATION OF THE COMPACTDRY EC CULTURE PLATES FOR THE DIAGNOSIS OF URINARY TRACT INFECTIONS IN HARARE, ZIMBABWE

Ioana Diana Olaru[1], Mutsawashe Chisenga[2], Rashida A. Ferrand[1], Wael Elamin[3], Shunmay Yeung[1], Heidi Hopkins[1], Prosper Chonzi[4], Katharina Kranzer[1]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*Biomedical Research and Training Institute, Harare, Zimbabwe,* [3]*King's College Hospital, Dubai, United Arab Emirates,* [4]*City of Harare, Health Department, Harare, Zimbabwe*

Data on antimicrobial resistance from low- and middle-income countries (LMICs) are limited. Reasons include poor laboratory capacity and stock management, and challenges meeting cold chain requirements. The CompactDry EC (CD, Nissui, Japan) culture system has a long shelf life (18 months from manufacture) and does not require refrigeration. It may thus be ideal for LMICs but has not been widely used for testing human samples. We describe the performance of CD for the diagnosis of urinary tract infections (UTIs) caused by *Enterobacteriaceae* in a LMIC context. A midstream urine sample was collected from adults presenting with UTIs to outpatient clinics in Harare and was inoculated on Brilliance UTI agar (Oxoid, UK), the reference standard. Growth was interpreted semi-quantitatively: 10^3-10^4 CFU/mL, 10^4-10^5 CFU/mL and >10^5CFU/mL. CD is chromogenic, supports growth of *Enterobacteriaceae*, and allows differentiation between organisms. Because CD is highly sensitive, urine samples were serially diluted at 1:10 for colony counts. CD was inoculated with 1 mL of the 1:10^3 and 1:10^6 urine dilutions. Of 140 samples 42 (30%), 95 (68%), 3 (2%) were positive, negative, and contaminated by reference standard. 27/42 (64%) samples grew *Escherichia. coli*, 4/42 (10%) other coliforms, and 11/42 (26%) gram-positive organisms (mainly enterococci). 29/31 (94%) of samples growing *Enterobacteriaceae* on the reference standard showed growth on CD at a dilution of 1:10^3. An additional 6 urines showed growth on CD (≤10 colonies/plate) but were negative by reference standard. The sensitivity of CD for *Enterobacteriaceae* was 98% (95%CI 92-99%) and the specificity 83% (95%CI 66-93%). There was a strong correlation between growth on the reference standard and the number of colonies on CD (Spearman's rho=0.74). This study shows that CD has a high sensitivity and specificity for the diagnosis of *Enterobacteriaceae*. CD has several advantages compared to conventional commercial media: long shelf life, storage at room temperature and small size of the plates. It may be a good alternative for surveillance of antimicrobial resistance in LMICs.

334

MOLECULAR TYPING OF *NEISSERIA GONORRHOEAE* ISOLATES FROM KENYA

Mary Wandia Kivata[1], Fredrick Lunyagi Eyase[2], Wallace Dimbuson Bulimo[2], Valerie Oundo[2], Esther Waguche[2], Wilton Mwema Mbinda[3], Margaret Mbuchi[2]

[1]*Karatina University, Karatina, Kenya,* [2]*U.S. Army Medical Research Directorate-Africa, Nairobi, Kenya,* [3]*Pwani University, Mombasa, Kenya*

Knowledge of circulating *Neisseria gonorrhoeae* (GC) strains, their antimicrobial resistance profiles, geographic distribution, and transmission patterns is crucial in the prevention and control of gonococcal infections. Molecular surveillance through sequence typing (ST) provides critical information on outbreaks and drug resistance. Due to inadequate GC surveillance in Kenya, data on the circulating GC STs are limited. The present study characterized circulating GC isolates from different regions in Kenya through molecular typing. Genomic DNA was extracted from 38 GC isolates and sequenced using the Illumina MiSeq. Annotation and genome analysis were performed using Bacterial Isolate Genome Sequence Database (BIGSdb) genomics platform tools. *N. gonorrhoeae* Multi Antigen ST (NG-MAST) was done at NG-MAST website while Multi-locus ST (MLST) was performed using MLST version 1.8. A total of 31 NG-MAST STs representing 23*porB* (10 novel) and 23 *tbpB* (7 novel) different alleles were identified. 33 (86.8%) isolates belonged to 27 novel NG-MAST STs while 5 (13.2%) isolates belonged to 4 previously reported STs. A novel NG-MAST, ST-19168, was the most prevalent (10.5%). 68.4% of the isolates formed singular NG-MAST STs. A total of 24 MLSTs representing 3 *abcZ*, 2 *adk*, 3*aroE*, 6 *fumC*, 5 *gdh*, 3 *pdhC*, and 2 different *pgm* alleles were identified. 25/38 isolates belonged to 14 known MLSTs STs while 15 belonged to 10 new MLSTs. 15 (39.4%) sequences formed single MLSTs. Core genome phylogeny revealed three distinct clusters characterized by varied MLST and NG-MASTs. Region based clustering was not observed. The observed diversity of MLSTs and NG-MASTs indicate that GC strains circulating in Kenya are genetically diverse. Although the present study is limited by the number of isolates analyzed, the observed non-regional distribution of both MLSTs and NG-MASTs indicate a heterogeneous gonococcal population in Kenya.

336

MATERNAL CHORIOAMNIONITIS AS A CAUSE OF PERINATAL DEATH IN THE CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE (CHAMPS) SITE OF MANHIÇA, SOUTHERN MOZAMBIQUE.

Rosauro Varo[1], Pio Vitorino[2], Antonio Sitoe[2], Elisio Xerinda[2], Marta Valente[1], Sara Ajanovic[1], Rita Mabunda[2], Dianna Blau[3], Khátia Munguambe[2], Inácio Mandomando[2], Quique Bassat[1]

[1]*Barcelona Institute for Global Health, Barcelona, Spain,* [2]*Centro de Investigação em Saúde de Manhiça (CISM), Manhiça, Mozambique,* [3]*Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, United States*

Chorioamnionitis is a common complication of pregnancy, associated with maternal morbidity and perinatal deaths. Its impact in low-income countries such as Mozambique is still poorly characterized. Between December 2016 and February 2019, as part of the Child Health and Mortality Prevention Surveillance (CHAMPS) network, minimally invasive tissue sampling (MITS) were conducted post-mortem in Manhiça district area to ascertain the cause of death of 171 children under five years of age and stillbirths, after written informed consent was obtained. Of those deaths, 117 (68.42 %) were classified as perinatal deaths: 59 stillbirths (34.5%), 41 deaths in the first 24 hours of life (23.97%), and 17 early neonatal deaths between 1 and <7 days of life (9.94%). For each case, clinical, microbiology, molecular, histo-pathological (including placenta whenever available) and verbal autopsy data were reviewed by a multidisciplinary panel of local experts who determined the chain of events leading to death and coded results using the WHO application of ICD-10 during the perinatal period (ICD-PM). In those 117 perinatal deaths, chorioamnionitis was included in the chain of events leading to death in 20 (17.09%) of the deaths. In 9/59 stillbirths (15.25%) and in 8/41 of the deaths occurring in the first 24 hours (19.51%) chorioamnionitis was identified as the main maternal condition. Three additional cases, one from each age group, included chorioamnionitis elsewhere in the chain of events leading to death. Chorioamnionitis was related to different infectious syndromes in the neonates such as pneumonia and sepsis. Most frequent pathogens isolated in tissue samples were *Streptococcus agalactiae* and *Streptococcus* species. Other microorganisms found were *E. coli, K. pneumoniae, P. aeruginosa* or *Sneathia amnii.* These data highlight the significant impact of chorioamnionitis on perinatal mortality. We will present a detailed analysis of the related demographic, maternal, clinical, microbiological and other data related to these deaths, and discuss implications for prevention strategies to enhance child survival.

337

RISK FACTORS AND OUTCOMES ASSOCIATED WITH INCREASED MORTALITY DUE TO CHOLERA INFECTION IN LMIC SETTINGS: A CASE FOR THE DOMINICAN REPUBLIC

Miguel A. Delgadillo

School of Medicine, Universidad Iberoamericana, Santo Domingo, Dominican Republic

With the on-going transmission and roughly 6,000 confirmed cases since 2012, Vibrio cholerae remains endemic within the Dominican Republic. Although the incidence rate in the Dominican Republic remains relatively low, large outbreaks with high case fatality ratios are increasing globally, making LMIC especially vulnerable to future epidemics. This study aims to evaluate risk factors associated with mortality in cholera infected populations during the period 2012-2018 in the Dominican Republic. Data detailing cases of cholera infections included in the Governmental Health Reports by the Ministry of Health and their demographic characteristics were solicited through their online forum. A total of 5345 cases of cholera infections were reported during the study period, with a mortality rate of 1.95% (n=104). Patients 20-29 years-old accounted for 19.9% (n=1066) of the total reports, being the most affected age group. Hospitalizations accounted for 89.9% (n=4803) of the total reports and ambulatory care was associated with twice the risk of mortality (p< 0.001). History of fever was observed in 14% of reports and is associated with hospitalization (OR: 1.7; p< 0.001). Males had a 2x increased risk of mortality (p=0.008). Pediatric patients were more likely than adult patients to be hospitalized due to cholera infection (OR: 1.6; p=0). The data demonstrates that male sex and receiving ambulatory care are two significant risk factors associated with increased mortality in cholera infected populations. Hospitals have a limited patient capacity making it extremely difficult to admit all patients suspected of cholera infection for treatment; however, patients with suspected cases of cholera infection must receive adequate care. We observed that 5.5% of females received ambulatory care, while 14.5% of males comprise ambulatory care. This discrepancy in the proportion of hospital admittance may explain why males resulted in a higher mortality rate. To lower cholera mortality rates, hospitalization guidelines should be further examined to encourage hospital admittance of individuals with suspicion of cholera infection.

339

USE OF NEXT GENERATION SEQUENCING IN DETERMINING NOSOCOMIAL SPREAD OF MDR BACTERIA IN A TERIARY HOSPITAL IN MANILA, PHILIPPINES

John Mark S. Velasco[1], Katie Margulieux[2], Ma. Theresa Valderama[1], Paula Corazon Diones[1], Cynthia Liao[3], Domingo Jr Chua[3], Fatima Claire Navarro[3], Patrick McGann[2], Brett Swierczewski[2], Anthony Jones[1], Stefan Fernandez[1]

[1]*US Army Medical Directorate-Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand,* [2]*Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [3]*V Luna Medical Center, Armed Forces of the Philippines Health Service Command, Quezon City, Philippines*

Carbapenemase-producing organisms harboring carbapenem resistance genes are leading causes of morbidity worldwide. We conducted biochemical identification and antimicrobial susceptibility testing using the BD Phoenix M50 at a hospital in Manila, Philippines from January 2014-December 2019. Imipenem, Meropenem or Ertapenem resistant isolates were screened for carbapenemase production using Carba NP. Carba NP positive isolates were tested for bla_{NDM}, and bla_{KPC} using real-time PCR. Isolates with negative PCR results were tested for $bla_{VIM,}$ $bla_{IMP-1,}$ and bla_{OXA-48} using the Cepheid Xpert Carba-R. Selected isolates were sent to WRAIR MRSN for short read whole genome sequencing using an Illumina MiSeq. Species identification was confirmed in silico, antimicrobial resistance genes present were investigated, and ad hoc analysis was performed to determine genetic relatedness of specific lineages and clones. Phylogenetic analysis were performed using RAxML from Panseq core genome alignment. ResFinder was used to predict antimicrobial resistance genes. Pairwise single nucleotide polymorphism analysis of core genome for clusters of highly related isolates was done to determine nosocomial transmission. Fifty-five unique isolates were sequenced with breakdown based on species, sequence type (ST) and important antibiotic resistance genes as follows: 3 *Escherichia coli* (ST-10, 156, 162; 1 bla_{NDM-1};2 *mcr-1.1*); 23 *Klebsiella pneumoniae* (ST-4,15,147,231,273,340,359,392; 5 bla_{KPC-2};8 bla_{NDM-1};9 bla_{NDM-7};1 *rmtC*); 21 *Acinetobacter baumannii* (ST-2,16,25,78; 18 *armA*;1 bla_{NDM-1};20 bla_{OXA-23};1 $bla_{OXA-420}$); 6 *Pseudomonas aeruginosa* (ST 235,3322,244,1212; 1 bla_{NDM-1};1 bla_{VIM-2});2 *Enterobacter cloacae* (ST-182,916; 2 bla_{NDM-7}). High genetic relatedness between pairs of isolates based on pairwise SNP analysis of core genome suggested a possible direct transmission of some isolates indicating potential nosocomial transmission or acquisition from a common reservoir. This underscores the utility of NGS in determining epidemiologic relationships of bacterial isolates and documenting occurrence of nosocomial spread.

340

METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*: HIGH PREVALENCE OF COMMUNITY-ASSOCIATED TYPES IN PATIENTS HOSPITALIZED AT A TERTIARY CARE HOSPITAL IN SOUTHERN SRI LANKA

Ruvini Kurukulasooriya[1], Bhagya Piyasiri[2], Weerasinghe M. D. G. B. Wijayaratne[1], Gayani Tillekeratne[3], Champika Bodinayake[1], A Dharshan de Silva[4], Bradly P Nicholson[5], Truls Østbye[3], Christopher Woods[3], Ajith Nagahawatte[1]

[1]*Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka,* [2]*Teaching Hospital, Karapitiya, Galle, Sri Lanka,* [3]*Duke University, Durham, NC, United States,* [4]*Faculty of Medicine, General Sir John Kotelawala Defence University, Ratmalana, Sri Lanka,* [5]*Institute for Medical Research, Durham, NC, United States*

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a pervasive multidrug-resistant pathogen. Based on epidemiological, genotypic, and antibiotic profiles, MRSA classifies as community-associated (CA) or healthcare-associated (HA). CA-MRSA is considered more aggressive due to its virulence factors. We investigated prevalence, risk factors and types associated with MRSA infection among patients admitted to Teaching Hospital Karapitiya (THK), a public, tertiary care hospital with 1500 beds. Consecutive *S. aureus* isolates were collected from September 2019 to January 2020 from the Clinical Microbiology Laboratory of THK. All *S. aureus* and MRSA isolates were confirmed by the laboratory using standard microbiological methods. Sociodemographic and clinical data were collected from medical records. Based on the antibiogram, isolates were characterized as HA-MRSA (resistance to ≥3 tested non-β-lactam antibiotics; ciprofloxacin, erythromycin, clindamycin, gentamycin and fusidic acid) or CA-MRSA, according to standard classifications. Sociodemographic factors associated with MRSA versus methicillin-susceptible *S. aureus* (MSSA) infection were accessed using Chi-square and Kruskal-Wallis tests in STATA version 13. A total of 203 *S. aureus* isolates from 190 patients were collected. Of all patients, 108 (56.8%) were male and 159 (83%) were adults (≥18 years). Isolates were obtained from blood (41, 20%), pus (120, 59%), respiratory (22, 10%), urine (11, 5%) and sterile fluid (9, 4%) cultures. MRSA was identified in 117 (57%) of *S. aureus* isolates, with most isolates cultured from pus (62%) and blood (16%). Out of all MRSA isolates, only 40 showed resistance to ≥3 tested non-β-lactam antibiotics, indicating CA-MRSA (65.8%) as the leading type of MRSA infection. Socio-demographic features such as age and sex were not significantly associated with MRSA infection. Majority of *S. aureus* isolated from clinical cultures at THK were MRSA. CA-MRSA can be recognized as the predominant type at THK and may cause more virulent infections. Further robust analyses including molecular data are needed to confirm the CA types.

341

ANTIMICROBIAL EFFECT OF O*CIMUM GRATISSIMUM, LAVANDULA ANGUSTIFOLIA* AND ACMELLA OLERACEA ON *STAPHYLOCOCCUS AUREUS* AND *PSEUDOMONAS AERUGINOSA* IN CALABAR, CROSS RIVER STATE, NIGERIA

Ofem Irom Ekpo, Regina Idu Ejemot-Nwadiaro, Hadiza Kubura Abdulsalam

University of Calabar, Calabar, Nigeria

Staphylococcus aureus and *Pseudomonas aeruginosa* infections account for majority of frequent cause of nosocomial infections globally with concomitant adverse consequences. One of such which is the increasing public health burden of rapid resistant of some strains of these microorganisms to antibiotics makes for search for antimicrobial agents from plant extract that could provide alternative ways of treatment of these infections. The antibacterial activities of *Acmella oleracea*, *Lavandula angustifolia* and *Ocimum gratissimum* plants against *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolates were determined in this study. The aqueous extracts from *Acmella oleracea*, *Lavandula angustifolia* and *Ocimum gratissimum* plants were extracted at concentrations of 100%, 50%, 12.5% by infusion method. Disc diffusion technique was used for testing for invitro antimicrobial activities of the 3 plant extracts against isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa,* while Ciprofloxacin and Tetracycline (0.25µg/m/ each) were used as controls to compare the standard antibiotic effect with that of the raw plant extracts for *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively. The sensitivity results show that antimicrobial effect against *Staphylococcus aureus* were demonstrated by *Acmella oleracea* extract at concentrations of 100% and 50% with zone of inhibition diameter (ZID) of 21mm and 15mm respectively; *Lavandula angustifolia* extract at concentrations of 12.5% with zone of inhibition diameter of 15mm while *Ocimum gratissimum* demonstrated low inhibition compared to 17mm observed for the standard Ciprofloxacin (ZID of 15 - 21mm indicates susceptibility while that < 6mm indicates resistance). No antibacterial activity against *Pseudomonas aeruginosa* were observed for the 3 plant extracts at all the concentrations studied (ZID <6mm). Invitro antibacterial activities exhibited by aqueous extracts of *Acmella oleracea* and *Lavandula angustifolia* against *Staphylococcus aureus* isolates suggests their potential use for *in vivo* treatment of these infections.

342

ANTIMICROBIAL RESISTANCE AND MOLECULAR CHARACTERISTICS OF EXTENDED SPECTRUM BETA LACTAMASE PRODUCING BACTERIA ISOLATED FROM DIABETIC FOOT INFECTIONS

Jeannette Bentum[1], Sarkodie Kodom[2], Stephen Simpeh[3], Patrick Feglo[2], Beverly Egyir[1]

[1]*Noguchi Memorial Institute for Medical Research, Bacteriology Department, Accra, Ghana,* [2]*School of Medical Sciences, College of Medical Sciences, KNUST, Kumasi, Ghana,* [3]*Department of Surgery, Korle-Bu Teaching Hospital, Accra, Ghana*

Common complications of diabetes mellitus include foot infections which are often polymicrobial in nature and involve multi-drug resistant bacteria such as Extended Spectrum Beta-Lactamases (ESBLs). In Ghana, routine detection of ESBLs is absent in most clinical microbiology laboratories; data on ESBLs recovered from diabetic foot infections is also scarce. Using phenotypic and molecular tools, this study therefore investigated the antimicrobial resistance trends of Gram negative isolates and ESBLs recovered from patients with diabetic foot infections. Tissue samples were collected from diabetic patients at the Korle-Bu Teaching Hospital. The isolates were identified using the MALDI-TOF. ESBL detection was done by plating isolates on ESBL Chrome agar. Antimicrobial susceptibility testing was performed by disk diffusion and interpreted by CLSI guideline. ESBL genes *(TEM, SHV* and *CTX-M)* were detected by PCR. In total, 142-Gram negative isolates were recovered from the 50 study participants. *E.coli* (27%) was the predominant bacteria specie recovered. Majority (99%) of the isolates were sensitive to meropenem and piperacillin-tazobactam and to gentamicin (97%). Resistance was recorded for cefuroxime (25%), cefotaxime (23%), chloramphenicol (23%) and tetracycline were 25%, 23%, 23% and 22% respectively. Forty-five (32%) isolates were positive for ESBL production. *CTX-M* gene was detected in 40% of the isolates, *TEM* gene in 22%; the *SHV* gene was detected in 20% of the isolates. The finding of ESBL positive isolates in the absence of routine detection of ESBLs in most clinical microbiology laboratories calls for laboratory capacity building for antimicrobial resistance testing and ESBL detection for effective patient management.

343

DIAGNOSIS OF ADVANCED HYPOTHYROIDISM INDUCED DILATED CARDIOMYOPATHY, A GLOBAL MEDICINE CASE STUDY

Daniel Haik[1], **Andrew Chunkil Park**[2], Andrew Siskind[2]

[1]*UC Irvine School of Medicine, Irvine, CA, United States,* [2]*UC Irvine Health, Orange, CA, United States*

Our patient is a 19 year old male from rural Guatemala with a longstanding history of undertreated hypothyroidism, resulting in dilated cardiomyopathy (DCM) with markedly-reduced ejection fraction as well as valvular dysfunction, a finding discovered utilizing point-of-care ultrasound. He presents to the clinic reporting persistent dry cough lasting 3 months. He went to the hospital recently where he was given an unknown injection, without noticeable improvement. He has been much more fatigued recently, in addition to gaining weight and feeling more cold than others do. No recent sick contacts or new environmental exposures were reported. The patient has been followed sporadically for over 13 years with poorly controlled hypothyroidism. His TSH values progressed from 26.75 µIU/ml measured 13 years ago, to greater than 75 µIU/ml measured 9 years ago, and then, to greater than 100 µIU/ml measured 7 years ago and at present. The patient's presentation at the hospital was his first endorsement of cardiac symptoms. His physical exam was notable for a systolic ejection murmur. Ultrasound demonstrated massively dilated left and right ventricles, with moderate mitral valvular regurgitation. Markedly reduced ejection fraction was noted, as was evidence of both systolic and diastolic dysfunction. Thyroid ultrasound demonstrated an atrophic thyroid. The patient was diuresed and started on more aggressive thyroid hormone replacement therapy with close follow-up scheduled. DCM secondary to hypothyroidism has been described in the literature, with reversal of cardiac disease demonstrated after sufficient thyroid hormone replacement. With adequate medication and close follow-up, this patient has a fair prognosis. However, a deeper understanding of the structural, cultural, and socioeconomic barriers present in providing and consuming healthcare in a low-resource context remains an essential component in ensuring effective care is being provided. In this case, limited access to transportation, patient education, and difficulty in attaining sufficient follow-up presented significant hurdles in maintaining consistent care.

345

DRAMATIC CHANGES IN BACTERIAL AND FUNGAL COMMUNITY DYNAMICS OVER THE FIRST FIVE YEARS OF LIFE IN THE MIDDLE-BELT OF GHANA, AFRICA

Dennis Adu-Gyasi[1], Nelly Amenyogbe[2], Yeetey Enuameh[3], Pinaki Panigrahi[4], Dennis Gyasi Konadu[1], Tobias Kollmann[5], Kwaku Poku Asante[1], Seth Owusu-Agyei[6]

[1]*Kintampo Health Research Centre, Kintampo North, Ghana,* [2]*Department of Experimental Medicine, University of British Columbia, Vancouver, BC, Canada,* [3]*Kwame Nkrumah University of Science and Technology, School of Public Health, Kumasi, Kumasi, Ghana,* [4]*Department of Pediatrics, Division of Neonatal and Perinatal Medicine, Georgetown University Medical Center, Washington, DC, United States,* [5]*Department of Pediatrics, University of British Columbia, Vancouver, BC, Canada,* [6]*University of Health & Allied Sciences, PMB31, Ho, Volta Region, Ghana, Ho, Ghana*

Introduction. Bacterial microbiomes are important in health and disease starting early in life already. Fungi also colonize our intestines early in life and also influence health and disease. Bacterial and fungal communities are known to interact intensely, and in this interaction along with the host determine the composition of the combined microbiome. We thus set out to both microbiome from birth over the first 2 years of life. *Methods.* We used 16S and ITS2 amplicon sequencing to measure bacterial and fungal microbiomes respectively and assessed alpha and beta diversity, community composition, and shared microbes between mother and infant pairs over the first 5 years of life. *Results*. While bacterial communities differed across the age spectrum in composition and diversity, the same was not observed for the fungal microbiome in parallel. We also observed a dramatic difference in maternal microbiomes between mothers of 0-5 and 26-35 day old infants. These changes included a higher abundance of *E. coli*, the most shared operational taxanomic unit (otu) between mother and infant stools, and lower abundances of *Prevotella* OTUs. Breastmilk microbiomes were dominated by bacterial (*Streptococcus, Staphylococcus*) and fungal taxa (*Malassezia*) common to skin. While infants shared more bacterial OTUs with their mother's stool and breastmilk than with unrelated pairs, there were far fewer fungal taxa consistently shared between infant and their mother's milk and almost none between infant and their mother's stool. Presence of Lp was confirmed in 29% of newborn samples, de-risking the potential use of this probiotic as an introduction of a foreign bacterial species to this population. *Conclusions.* Assessing fungal as well as bacterial community revealed several highly unique and important novel insights. We confirmed the well-described developmental trajectory of the microbiome in the developing infant gut.

346

PROSPECTIVE STUDY OF THE PERFORMANCE OF THE UNIVERSAL VITAL ASSESSMENT SCORE AND OTHER SEVERITY SCORES AMONG ADULT FEBRILE INPATIENTS IN NORTHERN TANZANIA, 2016-19

John P. Bonnewell[1], Deng B. Madut[1], Manuela Carugati[1], Michael J. Maze[2], Kajiru G. Kilonzo[3], Furaha Lyamuya[3], Annette Marandu[4], Nathaniel H. Kalengo[3], Bingileki F. Lwezaula[4], Blandina T. Mmbaga[3], Venance P. Maro[3], Matthew P. Rubach[1], John A. Crump[5]

[1]*Duke University, Durham, NC, United States,* [2]*University of Otago, Christchurch, New Zealand,* [3]*Kilimanjaro Christian Medical Centre, Moshi, United Republic of Tanzania,* [4]*Mawenzi Regional Referral Hospital, Moshi, United Republic of Tanzania,* [5]*University of Otago, Dunedin, New Zealand*

Early warning scores have been used to help improve triage of hospitalized patients in high-income settings, but such scores may not translate well to sub-Saharan Africa (sSA) due to epidemiological and healthcare-capacity differences in that setting. The Universal Vital Assessment (UVA) score, calculated based upon vital signs, mental status, percutaneous oximetry, and HIV-infection status, was developed as a clinical prognostic score for inpatient death among hospitalized patients in sSA. Our aim was to assess the performance of UVA and other prognostic scores, including the quick Sequential Organ Failure Assessment (qSOFA), Modified Early Warning Score (MEWS), and the National Early Warning Score (NEWS), for predicting in-hospital mortality in a cohort of adults with severe febrile illness in northern Tanzania. We enrolled patients admitted with febrile illness to two hospitals in Moshi, Tanzania from 2016-19. Performance characteristics, including sensitivity, specificity, and area under the receiver-operator characteristic curve (AUROC) for predicting in-hospital death were calculated for each score. We enrolled 617 adults aged ≥18 years. The median (IQR) age was 43 (31-56) years, 310 (50.2%) were female, and 207 (33.5%) were HIV-infected. In-hospital death occurred in 59 (9.6%) participants; 38 (64.4%) of these were in HIV-infected persons, conferring a 18.4% case-fatality ratio for this subgroup. AUROC was highest for UVA (AUROC 0.85, 95%CI 0.80-0.89), followed by NEWS (0.81, 0.75-0.87), MEWS (0.75, 0.66-0.78), and qSOFA (0.72, 0.66-0.78). While the performance of all scores decreased for HIV-infected participants, UVA (AUROC 0.75, 95%CI 0.66-0.84) had the highest AUROC value in that subgroup. In our cohort of febrile inpatients in northern Tanzania, UVA outperformed the other clinical scores in predicting in-hospital mortality. Our results suggest that UVA is a suitable clinical triage tool that could improve early recognition of febrile patients at high risk of in-hospital death in this setting. More operational research is needed to assess how such early recognition could improve patient management and outcomes.

347

A RARE CASE OF EUMYCETOMA OF THE HAND CAUSED BY THE PATHOGENIC MOLD *PHAEOACREMONIUM KRAJDENII* IN AN IMMUNOCOMPETENT PATIENT

Uli Schwab

Roayl Victoria Infirmary - Newcastle upon Tyne Hospitals, Newcastle upon Tyne, United Kingdom

Mycetoma, a Neglected-Tropical-Disease (NTD) with lacking accurate incidence/prevalence data, is a progressive destructive disease, assumed to be acquired by traumatic inoculation of certain fungi or filamentous bacteria, commonly involving the foot although any body part can be affected. A 44 year old man of Goan descent presents with 9 month of intermittent painful swelling of his left hand, partially responsive to NSAID's. He sustained a machete injury to the 2 proximal digit of his left hand 15 years prior, which rquired skin suitures. The dorsum of his left hand, particularly the 2nd Metacarpal (MC) bone was diffusely swollen and tender, with no erythema, sinus tract or skin changes. Normal inflammatory markers. Negative Blood borne virus screen. MRI revealed cortical thickening of the 2ndMC with enhancement of surrounding soft tissues suggestive of osteomyelitis. Open surgical bone/tissue biopsy sent for microscopy, culture and histology, yields a mycetoma on Grocott/DPAS staining; fungal hyphae with conidia are surrounded by eosinophilic-clubshaped-bodies with neutrophils adherent to the outer surface. Cous-Cous-like white grains discharged postoperatively from the wound and grew *Phaeoacremonium krajdenii* on (extended fungal culture) after 2 weeks, confirmed by 18s PCR. *Phaeoacremonium* species have shown *in vitro* susceptibility to a number of antifungal agents and he was started on oral posaconazole, which was well tolerated well, with good therapeutic clinical and radiological response. Characterization of the exact causative microbiological pathogen remains paramount but can be difficult, requiring invasive surgical sampling, dedicated culture and staining facilities. There are currently no established treatment breakpoints for filamentous fungi treatment. Eumycetoma often require prolonged treatment, which can be difficult, and unsatisfactory, even when the causative organism is identified. Azoles are used but are expensive, not always available, and may fail to eradicate the fungus, leading to recurrence or even amputation.

348

THE ADDITION OF LYMPHATIC STIMULATION ACTIVITIES TO A HYGIENE-CENTERED SELF-CARE PROTOCOL IMPROVES LYMPHEDEMA STATUS AMONG PEOPLE AFFECTED BY MODERATE TO SEVERE LYMPHEDEMA IN BANGLADESH

Jan Douglass[1], Hayley Mableson[1], Sarah Martindale[1], Sanya Tahmina[2], Mohammad Jahirul Karim[2], Muhammad Mujibur Rahman[2], Abdullah Al Kawsar[2], Abul Khair[2], Asm Sultan Mahmood[2], Akm Fazlur Rahman[3], Salim Mahmud Chowdhury[3], Susan Kim[4], Hannah Betts[1], Louise Kelly-Hope[1], Mark Taylor[1]

[1]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [2]*Ministry of Health and Family Welfare, Dhaka, Bangladesh,* [3]*Centre for Injury Prevention and Research Bangladesh, Dhaka, Bangladesh,* [4]*Flinders University, Adelaide, Australia*

Recommended management for lymphatic filariasis- related lymphedema is centred on a daily hygiene routine (standard-care) to reduce secondary infections. Adding lymphatic stimulating measures such as self-massage, drinking water, and deep breathing exercises to this routine (enhanced-care), may improve lymphedema status. The Indurometer is a handheld device with a 1cm diameter indenter applied to the skin under a 200g load. The Indurometer score quantifies tissue compressibility, an indicator of connective tissue change in lymphedema. A clinical trial compared enhanced-care (intervention) to standard-care (control) among people affected by moderate to severe leg lymphedema in Bangladesh. Cluster randomisation was used to allocate people to either the intervention or control groups, and Indurometer scores at the mid-calf of both legs were collected at 4- and 12-week follow-ups. There were 141 patients and Indurometer scores were available for 282 legs. At 4 weeks, scores ranged between 1.37 and 5.70 with significantly lower scores for legs affected by severe lymphedema (3.10 ± 0.91) compared to no lymphedema (3.49 ± 0.70), (adjusted difference of -0.31 (95%CI -0.55, -0.07), p=0.012) and there were no between-group differences (p=0.766). At 12 weeks, changes in tissue compressibility were greater among the intervention group compared to controls (intervention -0.58 (-0.71, -0.45), controls -0.32 (-0.45, -0.19), p<0.001)). The biggest change was observed on legs affected by severe lymphedema in the intervention group (-0.68 (-0.91, -0.45), p <0.001). Connective tissue change at mid-calf is increased when additional lymphatic stimulating activities are added to daily hygiene alone. Enhanced-care activities can be incorporated into existing morbidity management programmes, and data on tissue compressibility can be collected by health workers trained in Indurometry to objectively monitor lymphedema status and contribute to implementation research.

349

POSTNATAL OUTCOMES AND RISK FACTORS FOR IN-HOSPITAL MORTALITY AMONG ASPHYXIATED NEWBORN INFANTS IN A LOW-RESOURCE HOSPITAL SETTING IN NORTH-CENTRAL NIGERIA

Taofik Oluwaseun Ogunkunle[1], Halim Odiachi[1], Chuma Jerry Reuben[1], Surajudeen Oyeleke Bello[1], Abdulazeez Imam[2]

[1]*Dalhatu Araf Specialist Hospital, Lafia, Nigeria,* [2]*Department of Vaccines and Immunity, Medical Research Council Unit The Gambia at London school of Hygiene and Tropical Medicine, Fajara, Gambia*

Perinatal asphyxia currently accounts for about a-third of global newborn deaths and 95 percent of these deaths occur in low-resource settings. In Nigeria, asphyxia accounts for a significant proportion of neonatal admissions and case fatality rates are occasionally greater than 25 percent. Improvements in care of sick newborn infant in low-resource settings is key to reducing global newborn mortality and this would require requisite data on risk factors associated with mortality while in hospital care. In this study, we report outcome of newborn infants admitted to in-patient care in a resource poor setting in North-central Nigeria and document risk factors for mortality. We followed up a prospective cohort of 191 newborn infants admitted with perinatal asphyxia to a referral tertiary hospital in North-central Nigeria. Participants were followed up until mortality or discharge from hospital. At baseline, care-givers completed a structured questionnaire which was updated up until occurrence of our outcome. We compared baseline characteristics for participants who survived till discharge and those who did not using chi-square and independent t-test. We then fitted a multivariable logistic regression model to identify risk factors for mortality among the cohort. Majority (60.7%) of the participants presented to hospital within the first six hours of life. Despite this, mortality among the cohort was 14.7 percent with 32.1 percent dying within the first 24 hours of admission. Significant risk factors for mortality among the cohort were participant weight at admission (AOR = 0.11, 95% CI 0.03 to 0.40) and the presence of respiratory distress (AOR = 3.73, 95% CI 1.22 to 11.35). In conclusion, approximately two out of ten neonates with perinatal asphyxia died in the hospital in the current study. Low admission weight and respiratory distress were the significant predictors of in-hospital mortality.

350

QUALITY OF POSTNATAL CARE SERVICES IN ZAMBIA

Cephas Sialubanje[1], Nancy Scott[2], Lawrence Mwananyanda[3], Thandiwe Ngoma[3], Jeanette Kaiser[2], Rachel Fong[2], Davidson H. Hamer[2]

[1]*Levy Mwanawasa Medical University, Lusaka, Zambia,* [2]*Department of Global Health, Boston University School of Public Health, Boston, MA, United States,* [3]*Right to Care, Lusaka, Zambia*

Gaps in the quality of postpartum interventions contribute to poor maternal and newborn health outcomes globally. This study's aim was to evaluate quality of postpartum services women and their babies receive

and identify gaps in services provided. Four postpartum visits are required–within the first 24 h, 48-72 h, 7-14 days and 6 weeks. Exit interviews, developed according to the WHO quality of care framework, were conducted among 250 postpartum women from 12 health facilities in 3 districts of Southern Province, Zambia. Questions included demographic characteristics, place and duration of stay postpartum, waiting time, health provider skills and attitudes, type and perceived quality of services received. Data are expressed as adjusted odds ratios (aOR) and 95% confidence intervals (95%CI). Most participants (87.1%) gave birth in a health facility, half (51%) were observed for up to 24 hours and 58.6% stayed in a postnatal ward. Only 7.6% and 21.9% returned for their first and second visits as scheduled, and 33.3% at six weeks. Most (95.7%) were attended to by nurses; average waiting time was 25 minutes. Services provided included counselling on newborn care and nutrition (54%), BP measurement (86%), temperature check (7%), abdominal examination (49%), folic acid (54%) and iron supplementation (58%), tetanus toxoid injection (11%), and blood tests (28%). Laboratory tests included HIV (50%), hemoglobin (38%), syphilis (45%) and urine (29%). Only 12% of babies were fully examined and 10.5% had blood tests. Overall, 51% of the mothers perceived services to be low quality. Quality of care was positively associated with early postpartum visit (aOR=1.75, 95%CI: 1.01-3.03), number of services a woman received during a visit (aOR=1.28, 95%CI: 0.98-1.67) and if her baby was examined (aOR=3.85, 95CI: 1.47-10.1). Conversely, waiting time (aOR=0.99, 95%CI: 0.984-0.995) was negatively associated with postpartum care quality. These findings highlight limitations in quality of postpartum care provided in southern Zambia and identify major gaps including first visit timing, waiting time, number of services and quality of clinical examination.

352

INDIVIDUAL AND FAMILIAL CHARACTERISTICS OF PODOCONIOSIS PATIENTS ATTENDING A CLINIC IN MUSANZE DISTRICT, RWANDA

Jean Paul Bikorimana, Josee Dukuzimana

Heart and Sole Action, Musanze, Rwanda

Podoconiosis is a progressive, ascending lower extremity edema (swelling) that affects genetically susceptible people who live and walk barefoot on irritant red clay soils. The disease is considered a public health concern in many countries including Rwanda, where recent nationwide mapping documented podoconiosis in every district, with an average prevalence of 68 per 100,000. This study was retrospective to describe individual and familial characteristics of podoconiosis patients attending the Heart and Sole Action clinic in Musanze District, Rwanda. A total of 467 podoconiosis patient files were included and data was analyzed using industry recognized statistical software. The majority (375, 80.3%) of patients were female, with male to female ratio at 1:4.1. The mean age was 51.9 (SD=20) years and most (293, 62.7%) patients were aged above 45 years old. The mean age of podoconiosis onset among these patients was 34.4 years (SD=19.6) and many (139, 29.8%) developed podoconiosis when they were aged less than 20 years. Most of the patients (441, 94.4%) were farmers, and most (417, 89%) came from Musanze District or the neighboring Burera District, both in the Northern Province. More than half of the patients (238, 51.8%) had a family history of podoconiosis. When evaluating new podoconiosis patients, families with a higher number of podoconiosis diagnoses demonstrate an earlier age of onset compared to those with no or few relatives diagnosed with the condition (p&lt0.026). Given that recent nationwide mapping documented podoconiosis in every District of Rwanda, patients from only two districts make up the vast majority of those treated at Heart and Sole Action clinics (currently the only provider of care for podoconiosis patients countrywide). Our study suggests there is an urgent need to increase provision of care throughout the state system, in all districts. Given the strong familial patterns of disease reported, affected families must be supported to prevent new cases of podoconiosis among their unaffected children through use of preventive footwear and regular foot hygiene.

354

MINIMALLY INVASIVE TISSUE SAMPLING TECHNIQUE FOR DETERMINATION OF CAUSE OF DEATH AMONG NATURAL DEATHS IN GANDAKI PROVINCE OF NEPAL

Nuwadatta Subedi[1], Sunita Ranabhat[1], Madan Prasad Baral[2], Binita Koirala Sharma[1], Suraj Bhattarai[1]

[1]*Gandaki Medical College Teaching Hospital and Research Center Pvt Ltd, Pokhara, Nepal,* [2]*Pokhara Academy of Health Sciences, Western Regional Hospital, Pokhara, Nepal*

There are several constraints to complete diagnostic autopsy in Nepal. The study is conducted with objectives to identify existing diseases and conditions and to determine the cause of death using Minimal Invasive Tissue Sampling (MITS) technique followed by microbiological and histopathological tests. The study cases comprise of adult bodies in natural deaths. This is an ongoing study and we have presented findings from first 30 cases. The ante mortem and demographic information are entered in the standard case report form (CRF). Postmortem collection of specimens is done using MITS technique. The samples are undergone serological, microbiological and histopathological tests to detect pathogens and underlying pathology. Among 30 cases, 24 were males and six females with age range 20 to 76 years. Dengue IgM and *Brucella Abortus* antigen were positive in four cases each. Malaria (Pf, Pan Malaria) and HIV1 was reactive in one case each. Blood culture results detected Methicillin sensitive *S. aureus* in five cases and *Klebsiella pneumonia* in two. *Klebsiella oxytoca* and *Escherichia coli* were detected by CSF culture in one case each. Lung tissue culture detected *Klebsiella pneumonia* in eight cases, *Acenetobacter Calcoaceticus* Complex, *Citrobacter fruendii*, Methicillin sensitive *S. aureus, Klebsiella oxytoca,* and *Pseudomonas aeruginosa,* in two cases each. *Mycobacterium tuberculosis* was detected from GeneXpert test in two cases. Lung tissue histopathology diagnosed Anthracosis in 14 cases, bronchopneumonia in four, interstitial pneumonitis in two, adenocarcinoma and bacterial colony in one case each. Liver tissue histopathology showed steatosis in eight cases, steatohepatitis in three and metastatic deposit of tumor in two cases. Minimal Invasive Tissue Sampling (MITS) technique and further serological, microbiological and histopathological tests has been useful to detect underlying pathology for the determination of cause of death in our setting.

355

LACTULOSE, RHAMNOSE ASSAY OF INTESTINAL PERMEABILITY AS A NON-INVASIVE TEST FOR DETECTION OF ENVIRONMENTAL ENTERIC DYSFUNCTION IN YOUNG CHILDREN

Zehra Jamil[1], Najeeha Talat Iqbal[1], Kamran Sadiq[1], Romana Idress[1], Zubair Ahmad[1], Sheraz Ahmed[1], Fayaz Umrani[1], Sana Syed[2], Kumail Ahmed[1], Junaid Iqbal[1], Sean Moore[2], Asad Ali[1]

[1]*Aga Khan University, Karachi, Pakistan,* [2]*University of Virginia, Charlottesville, VA, United States*

Environmental Enteric Dysfunction (EED), an acquired small intestinal condition is a precursor of growth faltering in children living in low- and middle-income countries. Frequent exposure to enteropathogens induces persistent inflammation and damage to the gut epithelium resulting in decreased functional surface area and increased permeability in children living in impoverished conditions. Histologically, it is characterized by villous atrophy, crypt hyperplasia and immune cells infiltration. The lactulose-rhamnose (LR) dual sugar absorption test is a non-invasive indicator of gut barrier function and surface area. Our study explored LR absorption as a biomarker of growth faltering and EED severity in 63 undernourished children who underwent both LR testing as well as upper endoscopy and 51 healthy controls who received the LR test alone. Mean height-for-age Z (HAZ) at 24 months for cases was -2.90 (SD=1.14) vs. -1.45 (SD=1.14) for controls. The mean (SD) age in months at the time of assay was similar between cases and controls [14.17 (2.48) vs 14.14 (2.02)]. Cases had relatively lower sugar absorption than controls, with

median (Q1,Q3): 27 (11.5, 59.5) vs. 37 (12, 61) ug/ml for lactulose, and 60 (28, 178) vs. 86 (29, 170) ug/ml for rhamnose, but the differences were not statistically significant. The median LR ratio of cases and controls was 0.47 & 0.52 respectively (p>0.05). There was no significant correlation between sugar levels and growth parameters (p>0.05); however, reduction in the goblet cell density negatively correlated with lactulose (-0.371, p=0.014), rhamnose (-0.355,p=0.006), and LR ratio (0.211, p=0.109). Lactulose and rhamnose were marginally associated with HAZ at 24 months in multivariable regression after adjusting for other potential confounders. Mucins and other molecules secreted by goblet cells maintain intestinal mucosal homeostasis. Defective mucous barrier may be associated with inflammation and increased permeability of the gut lining. We conclude that in setting with a high prevalence of stunting, the LR test should be cautiously used as a biomarker for barrier function in EED suspected children.

356

CLINICAL PREDICTORS OF SEVERE DEHYDRATION IN ADULTS AND OLDER CHILDREN WITH ACUTE DIARRHEAL DISEASE IN BANGLADESH

Meagan A. Barry[1], Monique Gainey[2], Sabiha Nasrin[3], Md. Nur Haque Alam[3], Eric J. Nelson[4], Christopher H. Schmid[1], Stephanie Garbern[1], Kexin Qu[1], Adam C. Levine[1]

[1]*Alpert Medical School of Brown University, Providence, RI, United States,* [2]*Rhode Island Hospital, Providence, RI, United States,* [3]*International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh,* [4]*University of Florida, Gainesville, FL, United States*

Diarrheal disease is the 5th leading cause of years of life lost globally. Patients with aggressive disease leading to severe dehydration require rapid resuscitation with intravenous fluids and hospitalization, while patients with milder dehydration can be treated with oral rehydration solution as outpatients. Currently, no validated clinical prediction models for dehydration in adults or older children with diarrhea exist. This study seeks to determine clinical predictors of severe dehydration in adults and older children with diarrhea, to better triage and treat these patients. A prospective cohort study was conducted of adults and children over five years presenting with acute gastroenteritis to the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), March 2019–January 2020. Percent dehydration was calculated as the percent difference between patient's dehydrated weight on presentation and post-hydration stable weight, and categorized as severe (9%), or some (<9%) dehydration. Various clinical parameters were measured and logistic regression employed to assess association between each predictor and severe dehydration. Of 1,921 patients enrolled who obtained a stable post-hydration weight, 256 (13%) had severe dehydration. Clinical parameters associated with severe dehydration included minimal/no urine output (OR=2.81, 95% CI 1.53-5.17), confused/lethargic mental status (OR=2.21, 95% CI 1.34-3.64), slow (OR= 4.54, 95% CI 3.06-6.74) or very slow (OR=10.7, 95% CI 6.83-16.8) skin pinch, sunken eyes (OR=3.06, 95% CI 2.06-4.55), deep respirations (OR=3.39, 95% CI 2.45-4.68), decreased (OR=2.39, 95% CI 1.69-3.37) or absent (OR=5.41, 95% CI 2.75-10.65) radial pulse, MUAC<220 (OR:2.91 95% CI 2.22-3.82), and MAP<60 (OR=2.46, 95% CI 1.84-3.30). This study finds several clinical parameters predictive of severe dehydration in adults and older children with diarrhea. Future research could utilize these parameters to develop and validate a clinical prediction model to help clinicians rapidly identify severely dehydrated patients, and thus guide treatment approach.

357

LEVEL OF LITERACY AND CLINICAL OUTCOMES IN PATIENTS WITH CHAGAS DISEASE: SAMI-TROP PROJECT

Clareci Silva Cardoso[1], Nayara Dornela Quintino[1], Gabriela Lemes David[1], Ester Cerdeira Sabino[2], José Luiz Padilha da Silva[3], Antonio Luiz Pinho Ribeiro[4], Ariela Mota Ferreira[5], Lea Campos Oliveira[2], Claudia Di Lorenzo Oliveira[1]

[1]*School of Medicine, Federal University of São João del-Rei, Divinópolis, Brazil,* [2]*Institute of Tropical Medicine, University of São Paulo, São Paulo, Brazil,* [3]*Department of Statistics, Federal University of Paraná, Curitiba, Brazil,* [4]*Clinical Hospital and School of Medicine, Federal University of Minas Gerais, Belo Horizonte, Brazil,* [5]*Clinical Hospital, State University of Montes Claros, Montes Claros, Brazil*

Chagas disease (CD) is a neglected tropical disease that affects mainly vulnerable population, whose majority has low ability to understand health information. This study aimed to assess the prevalence of health literacy (HL) and its association with sociodemographic, quality of life, health care aspects and worse clinical outcomes. This is a cross-sectional study developed inside a cohort study (SaMi-Trop) including 1959 patients. It has been conducted in an endemic region to CD in Brazil. The eligible criteria for HL evaluation was the ability to read. The HL was assessed with SALPHA-18 scale and literacy was categorized in inadequate HL; adequate HL and; illiterate. Multiple models were adjusted using binary logistic regression, multinomial and beta regression models using the gamlss framework. Of the patients included, 1136 (74.1%) are illiterate. For HL assessment, only 397 managed to complete the HL evaluation. The prevalence of inadequate HL was 85.1% (338), only 59 patients (14.9%) had adequate HL. Our results are as following: 1) being illiterate increases the chance of using more medicines when compared to individuals with adequate HL - 1 or 2 medicines (OR: 1.96; CI: 1.06-3.62) and 3 to 4 medications (OR: 3.06; CI:1.44-6.52), to have hypertension (OR: 2.24; CI: 1.29-3.90), report an average self-perceived health (OR: 2.97; IC: 1.63-5.42), report poor self-perceived health (OR: 3.67; CI: 1.71-7.89) and have Chagas cardiomyopathy with left ventricular disfunction (OR: 3.23; IC:1.28-8.17); illiterate patients present worst quality of life scores in Physical (OR: 0,.73; CI: 0.58-0.91), Psychological (OR: 0.67; CI: 0.54-0.83) and Environmental (OR: 0.73; CI: 0.60-0.88) domains when compared to individuals with adequate HL. 2) inadequate literacy increases the chance of using 3 to 4 medications (OR: 2.26; CI: 1.04-4.93), report an average self-perceived health (OR: 2.48; CI: 1.34-4.62) and have Chagas cardiomyopathy with left ventricular disfunction (OR: 2.59; IC: 1.00-6.70). We found a high prevalence of inadequate HL that was associated with worse clinical outcomes and poor self-perceived health.

358

DETECTION OF *TLR9* POLYMORPHISMS AND THEIR ASSOCIATION WITH SEXUALLY TRANSMITTED INFECTIONS, CERVICITIS AND CERVICAL CANCER

Alex Chauhan[1], Nilesh Pandey[2], Neeraj Jain[2]

[1]*Brock University, St. Catharines, ON, Canada,* [2]*Charotar University of Science and Technology, Anand, India*

Cervicitis is characterized by the inflammation of cervix and is mainly caused by infection of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG). Infection of *Trichomonas vaginalis* (TV) and HPV are also considered as important factors of cervicitis. High-risk HPV infection is a well-known etiologic agent of cervical cancer; moreover, persistent inflammation is also an important predisposing factor of carcinogenesis. In addition, polymorphisms in Toll-like receptor (TLR) genes have been associated with pathogen infection and disease development including cancer. TLR9 is known to recognize DNA of pathogens. Variations in the TLR9 genes are known to influence susceptibility to infections as well as disease progression. The present study was designed analyse five common *TLR9* SNPs to identify their potential as susceptibility marker. The study included 130 cervicitis cases with 150 age-matched controls and 110 cervical cancer patients with 141 age-matched controls. CT, NG, TV and

HPV were detected in cervicitis whereas twelve high-risk HPV types were detected in cervicitis and cervical cancer cases, in addition to all the control subjects, using the using real-time PCR. Five common TLR9 SNPs were analysed using PCR-RFLP or allele specific-PCR. Haploview and Locusview were employed for linkage and haplotype analysis. TV infection was found at highest frequency (30.7%) as compared to CT (1.5%), NG (2.3%) and HPV (4.6%) infections in cervicitis patients. In the case of cervical cancer, hr-HPVs were detected in 81.6% patients. HPV16 and 18 was detected in 64% and 3.6% cervical cancer cases, while the frequency of HPV 45 was 13.6%. The genotypes TC (rs187084) and TC (rs5743836) as well as C (rs5743836) allele were marginally associated with TV infected cervicitis patients. The genotypes TC and CC as well as C allele of rs187084 polymorphism served as risk factors for cervical cancer. The haplotypes GTA was found to be associated with cervicitis, CTGA with TV infected cervicitis, and GATC with cervical cancer. *TLR9* polymorphisms and haplotypes were significant associated in modulating risk to cervicitis and cervical cancer.

359

CHARACTERIZATION OF HUMAN IMMUNOGLOBULIN PRODUCT GENERATED IN A MODULAR MANUFACTURING UNIT USING A RAPID MANUFACTURING PROCESS

Wesley deLeeuw, **Tharmala Tharmalingam**, Patrick Wiebe, Aynslie Wall, Evelyn Van der Hart, Shelly Buhay

Emergent Biosolutions, Winnipeg, MB, Canada

A rapidly deployable drug product for use in the early stages of a disease outbreak could prevent further spread of the pathogen. Emergent BioSolutions is developing a rapid response platform (RRP) to manufacture human immunoglobulins (IgG) for use as a passive immunotherapy during public health emergencies. The RRP begins with identification of plasma donors with measurable levels of pathogen-specific antibodies using a field deployable real-time donor screening assay. Plasma collected from eligible donors is treated using Mirasol® Pathogen Reduction Technology, pooled, purified, and concentrated/formulated using Emergent's rapid IgG manufacturing process. The 30 L process aims to generate ~65 doses of IgG drug product (1 g of total IgG/dose) with testing and release onsite for immediate use. The IgG product is manufactured onsite in a modular manufacturing unit (MMU), which houses a self-contained manufacturing environment and all required infrastructure within a 53-foot shipping container. Leveraging Emergent's experience in hyperimmune manufacturing and purification platform technologies, the process has been designed to be feasible within a significantly smaller footprint while yielding a product with quality attributes that meet compendial specifications for an IgG intended for intramuscular administration. In this poster, we will present analytical data from development runs, including molecular size distribution (size exclusion chromatography) and purity (agarose gel electrophoresis). We will also demonstrate substantial reduction of riboflavin and its degradants (process impurities introduced by the Mirasol pathogen reduction treatment). This data will support the quality and purity of the IgG product produced within the MMU using Emergent's rapid IgG manufacturing process.

360

CO-INFECTION OF MALARIA AND BACTERIAL PATHOGENS AMONG PATIENTS PRESENTING WITH FEBRILE ILLNESS IN GHANA

Yeboah Clara[1], **Behene Eric**[1], Kumordjie Selassie[1], Tagoe Janice[1], Nimo-paintsil Shirley[2], Attram Naiki[2], Letizia Andrew[3], Fox T. Anne[2], Watters Chaselynn[2], Sanders Terrel[2], Wolfe David[2]

[1]*Noguchi Memorial Institute For Medical Research, Accra, Ghana,* [2]*Naval Medical Research Unit-Three, Ghana Detachment, Accra, Ghana,* [3]*Infectious Disease Directorate, Naval Medical Research Centre, Silver Spring, MD, United States*

Malaria has for many years been the leading cause of fever in the African sub-region. However, improved control measures have significantly contributed to the huge decline in the incidence of the disease. Misdiagnosis of non-malarial aetiologies may be attributed to the overlap of their symptoms with malaria. This worrying trend demonstrates the importance of further investigations into non-malarial fever cases, which account for about 50% of all fever presenting morbidities in sub-Saharan African region. This study sought to understand the dynamics of the possible aetiologies causing febrile illness in Ghana, and to determine the coinfection between malaria and other bacterial infections in febrile patients.Participants were enrolled between July 2015 and December 2019 from 4 Ghanaian military treatment facilities. Inpatients or outpatients, 30 days to ≤65 years of age, with documented or reported fever (> 38°C) were eligible for the study. In addition to clinical and demographic information, blood specimens were collected for culture, serology and molecular testing.The 399 febrile participants, 176 (53.2%) male and 155 (46.8%) female, presented with similar clinical symptoms including fever, chills, headache, muscle and joint pain. Malaria was diagnosed in 275 (68.9%) participants and, of these, co-exposure was noted to leptospirosis (5.3%) and Q-fever (11.7%). For samples cultured, bacterial co-infection was noted in 6/164 (3.7%) of the malaria positive samples. Among the 124 malaria negative participants, 4.1%, 8.1% and 6.8% were exposed to leptospirosis, Q-fever and bacterial pathogens from culture, respectively. None of the presenting signs and symptoms had a significant association with the diseases except for headache which was significantly associated with bacterial pathogens from culture ($p<0.005$).An understanding of the dynamics of malaria co-infection and the possible contribution to febrile infections will guide diagnosis and treatment, improve clinical outcomes, and advance knowledge of the disease state for health care providers.

361

LEVERAGING TECHNOLOGY TO ADDRESS THE NEGLECTED PROBLEM OF UNDIAGNOSED HEARING LOSS IN SUB-SAHARAN AFRICA

Samuel Ficenec[1], Latha Rajan[2]

[1]*Tulane School of Medicine, New Orleans, LA, United States,* [2]*Tulane School of Medicine and Tulane School of Public Health and Tropical Medicine, New Orleans, LA, United States*

In a rousing call to action to address the rising prevalence of hearing loss (HL), the WHO highlighted it as one of the leading causes of disability worldwide, with 18.7% of global population living with HL. They further state that the prevalence of disabling hearing loss (DHL) is almost 500 million. The longitudinal growth of DHL is predicted to reach a billion by 2050 with demographic changes. However, even minimal levels of HL lead to chronic socioeconomic detriments among both adults and children. Most importantly, prevalence increases exponentially as national income decreases, with LMICs emphatically demonstrating this linear relationship by having 80% of the burden. One of the highest impacted regions is Sub-Saharan Africa (SSA). Compounding the problem is lack of access to specialist healthcare and gaps in testing skills and infrastructure. The gold standard diagnosis requires an audiometric exam conducted by an audiologist in a sound-proof booth, which is both labor cost and prohibitive in many world regions. In the face of such dramatic deficiencies, we investigate whether technology could be the answer. The simplest and most practical of these interventions is the smartphone, a technology that has become widespread, even in low resource settings. We undertook this study to assess the feasibility, challenges and cost-effectiveness of mobile and tablet-based audiometry applications for diagnosis of HL in SSA. After eliminating studies that lacked necessary data, studies using a total of 7 commercially available mobile audiometry applications were narrowed down and analyzed. The median sensitivity of all mobile applications was 82.42% (range= 0-100) and the median specificity of mobile applications was 85% (0-100). The cost of the commercially available applications ranged from $0-$4100 USD (median = $38.99, $p<0.001$). Some of the solutions can be cost-effective and implemented as priority. The evidence presented in this study will prove useful in guiding the adoption of mobile health applications as a cost-effective intervention for early diagnosis of HL and early linkage to care to prevent HL and other complications.

362

ACUTE FEBRILE ILLNESS IN TWO RURAL BORDER PROVINCES, THAILAND, 2017-2019

Saithip Bhengsri[1], Christopher J. Gregory[2], Pongpun Sawatwong[1], Sumonmal Uttayamakul[3], Ornuma Sangwichian[1], Nuttagarn Chuenchom[4], Kittisak Tanwisaid[5], Tuwan Simmali[6], Sumpun Butapun[7], Suttinee Boonlong[7], Barameht Piralam[6], Samkan Chaoprasert[7], Toni Whistler[8], Beth Skaggs[1], Emily Bloss[1], John R. MacArthur[1]

[1]*Division of Global Health Protection, Centers for Disease Control and Prevention, Nonthaburi, Thailand,* [2]*Arboviral Diseases Branch, National Center Emerging and Zoonotic Infectious Diseases, Fort Collins, CO, United States,* [3]*Bamrasnaradura Infectious Disease Institute, Nonthaburi, Thailand,* [4]*Maesot Hospital, Tak, Thailand,* [5]*Nakhon Phanom Hospital, Nakhon Phanom, Thailand,* [6]*Provincial Health Office, Nakhon Phanom, Thailand,* [7]*Provincial Health Office, Tak, Thailand,* [8]*Division of Global Health Protection, Centers for Disease Control and Prevention, Atlanta, GA, United States*

Acute febrile illness (AFI) can be caused by many different pathogens. The etiologies of AFI in Thailand remain largely unknown, due in part to a lack of reliable or accessible laboratory diagnostics. We describe the etiologies for AFI patients hospitalized with undifferentiated fever (UF) and estimate the disease burden in two rural Thailand provinces, Nakhon Phanom and Tak. Specimens were collected from AFI patients aged 2-80 years with UF; defined as (a) a temperature >38ºC or (b) history of fever <7 days and no evidence of >2 respiratory tract symptoms nor diarrheal disease. Demographic information and laboratory results were abstracted from medical records. Patients were interviewed for clinical symptoms and risk factors. Blood samples were collected for culture, rapid diagnostic tests (RDT) and molecular testing. During April 2017 - December 2019, 10,801 of 20,502 (53%) screened patients were eligible for inclusion into the study. Of these, 26% (2,801/10,801) were classified as having UF. Preliminary results indicated 39% (1,104/2,801) of patients with UF had pathogens identified. Among hemoculture positive UF patients, the three most common pathogens isolated were *Escherichia coli* [119/264 (45%)], followed by *Burkholderia pseudomallei* [21/264 (8.0%)] and *Klebsiella pneumoniae* [21/264 (8.0%)]. For RDT results, dengue NS1 antigen and/or IgM antibodies were positive in 18% (489/2,725), *Orientia tsutsugamushi* IgM was positive in 3.5% (95/2,725) and pan-malaria was positive only in Tak in 1.6% (25/1,595) of patients tested. PCR results indicated dengue-2 [118/297 (40%)] and dengue-1 [93/299 (31%)] were the most common serotypes in Nakhon Phanom and Tak, respectively. Chikungunya, pathogenic *Leptospira* species, *Rickettsia* species, *B. pseudomallei* and Zika virus were detected in 4.7% (125/2,667), 2.7% (71/2,668), 2.6% (70/2,668), 0.4% (12/2,672) and 0.1% (3/2,638) among those PCR positive. Vector-borne and zoonotic diseases, as well as invasive bloodstream infections are important causes of UF illnesses in two rural border provinces in Thailand, yet the etiologies for over half of the cases remain unknown.

363

ASSESSMENT OF CLINICAL REFERENCE RANGES OF PULSE RATE, RESPIRATORY RATE, BLOOD PRESSURE, TEMPERATURE AMONG HEALTHY SUBJECTS ATTENDING THE SCREENING FOR MALARIA VACCINE IN BIOKO ISLAND, EQUATORIAL GUINEA

Luis R. Abaga[1], Maria Silvia Lopez[2], Said Jongo[3], Vicente Urbano Nsue Ndong[2], Raul chuquiyauri[1], Ali Hamad[3], Beltran Ekua Ntutumu[2], Wonder Philip Phiri[1], Kamaka Ramadhani Kassim[3], Martin Eka Mangue[2], Genaro Nsue Nguema[2], Thabit Mbaga[3], Julia Yumbe Baka[1], Elizabeth Nyakarungu[3], Thomas Richie[4], Guillermo Garcia[1], Ines Toichoa[1], Godwin Fuseini[1], Stephen L. Hoffman[4], Olivier Tresor[1], Maximillian Mpina[5], Jose Raso[2], Preston Church[4], Peter Billingsley[4], Salim Abdulla[3]

[1]*Medical Care Development International, Malabo, Equatorial Guinea,* [2]*Ministry of Health and Social Welfare, Malabo, Equatorial Guinea,* [3]*Ifakara Health Institute, Dar es salaam, United Republic of Tanzania,* [4]*Sanaria Inc, Rockville, MD, United States,* [5]*Swiss Tropical and Public health institute., Basel, Switzerland*

Clinical decision making in the clinical trial or medical care relies on the history, examination, and results of selected investigations. As part of the general clinical examination, four clinical parameters are routinely recorded: heart rate, respiration rate, blood pressure, and temperature. Accurate interpretation of these parameters is paramount important during screening of participants for eligibility check and safety follow up in the clinical trial as well as during disease screening, diagnosis, monitoring disease progression, and treatment efficacy. The recorded clinical parameters must be compared with a reference range in order to derive meaningful information. The clinical parameters ranges may vary in individual genetic, ethnic and socio-economic factors. Based on these variations, reference ranges developed for one particular population may not be applicable to others. Therefore, it is imperative that reference ranges are accurate and reduce the risk of incorrect assessments of these parameters. The aim of this study is to establish a reference range of heart rate, respiratory rate, blood pressure, temperature in Equatorial Guinea. The design of the study is a cross-sectional study. The data will be extracted from the ongoing study titled Pilot Study to Optimize Recruitment and Screening Procedures for Future Clinical Trials and to Create a Registry of Potential Research Participants on Bioko Island, Equatorial Guinea. The pilot study is expected to recruit 3500 healthy males and females aged 18 months to 50 years old living in selected areas of Bioko Island with high malaria transmission. The study started in September 2019 and final monitored data is expected to be available in July 2020.Calculation of reference intervals will be performed using the 2.5^{th} and 97.5^{th} centile of the distribution with 90% CI based on Clinical and Laboratory Standards Institute guidelines. The analyte Will be stratified by age groups and gender. The analysis of outlier will be performed by using D test (one third rule).

364

REFERENCE RANGES, CHARACTERISTICS OF THE ELECTROCARDIOGRAM FINDINGS AND ITS IMPLICATION TO THE SCREENING AND RECRUITMENT FOR MALARIA CLINICAL TRIAL PARTICIPANTS IN BIOKO ISLAND, EQUATORIAL GUINEA

Gertrudis Owono Bidjimi[1], Maria Silvia Angue[1], Kamaka Ramadhani Kassim[2], Vicente urbano Nsue Ndong[1], Thabit Mbaga[2], Beltran Ekua Ntutumu[1], Ali Hamad[2], said Jongo[2], Wonder Philip Phiri[3], Julia Yumbe Baka[3], Ines Toichoa[3], Olivier Tresor[3], Godwin Fuseini[3], Guillermo Garcia[3], Juan Carlos Momo[1], Matilde Riloha Rivas[4], Raul Chuquiyauri[3], Preston Church[5], Peter Billingsley[5], Maximillian Mpina[6], Elizabeth Nyakarungu[2], Salim Abdulla[2], Kamaka Kassim[2], Thomas Richie[5], Stephen L. Hoffman[5]

[1]*Ministry of Health and Social Welfare, Malabo, Equatorial Guinea,* [2]*Ifakara Health Institute, Dar es salaam, United Republic of Tanzania,* [3]*Medical*

Care Development International, Malabo, Equatorial Guinea, [4]Ministry of Health and Social Welfare, Dar es salaam, Equatorial Guinea, [5]Sanaria Inc, Rockville, MD, United States, [6]Swiss Tropical and Public Health Institute, Basel, Switzerland

The electrocardiogram (ECG) is a globally used essential, inexpensive, and non-invasive technique to detect electric abnormalities of the heart, information for ECG values in a healthy cohort is of major importance to distinguish between average values of the ECG, but most of these studies focused on a specific subpopulation, such as athletes or an elderly population, or included only women. Furthermore, previous studies showed that interpretation of the ECG should be corrected for age categories, sex, and ethnicity. Therefore, there is a need to carry out a study in Equatorial Guinea to establish the local reference range of the electrocardiogram (ECG). To present reference ranges, characteristics of the electrocardiogram findings and its implication to the screening and recruitment for Malaria clinical trial participants in Bioko Island Equatorial Guinea. The design of the study is cross-sectional. The ECG data collected during the implementation of the ongoing study with the title "pilot study to optimize recruitment and screening procedures for future clinical trials and to create a registry of potential research participants on Bioko Island, Equatorial Guinea" will be analyzed. The population of the pilot study is expected to be 3500 health individuals aged 18 months to 50 years old living in selected areas of Bioko Island with high malaria transmission. The following six ECG parameters will be analyzed to find out the reference range based on age and sex, Heart rate, PR Interval, QRS duration, QTc Interval, QRS axis, sv1+rv5 amplitudes. The reference value will be estimated using non- parametric methods. The 2.5 and 97.5 percentiles will be computed for each parameter. The analysis of the outlier will be performed by using the D test (one-third rule). The study started in September 2019 and final monitored data is expected to be available in July 2020.

365

MINIMALLY INVASIVE TISSUE SAMPLING ACCEPTABILITY AT KIGALI UNIVERSITY TEACHING HOSPITAL, RWANDA

Ntakirutimana Gervais

Kigali University Teaching Hospital, Kigali, Rwanda

Postmortem data remains almost inexistent in resource limited countries including Rwanda. This makes prioritization in health system difficult. We assessed qualitative variables to evaluate the level of acceptability of Minimally Invasive Tissue Sampling (MITS) for patients dying at CHUK. An institution review board approval, consent form from next of kin of deceased were sought. Ninety six percent (96 %) of interviewed health professionals and relatives of the deceased accepted the Minimally invasive Tissue Sampling. In conclusion, Minimally invasive Tissue Sampling is a reliable and acceptable Method that can be alternative of complete Autopsy to generate mortality data in Rwandan population.

366

STABILITY OF CHOLERA VACCINE CVD 103-HGR UNDER VARYING ENVIRONMENTAL CONDITIONS

Paul R. Duffin, Amish Patel

Emergent Biosolutions, Gaithersburg, MD, United States

The attenuated recombinant *Vibrio cholerae* O1 strain CVD 103-HgR, redeveloped as PVXV0200, is supplied as one buffer packet and one active vaccine packet which are mixed with water and ingested. It is stored at 2-8ºC and consumed within 15 minutes of reconstitution. Methods: To assess the stability of PXVX0200 beyond the current prescribing parameters, vaccine potency was measured under a variety of environmental conditions. Vaccine and buffer packets were removed from 2-8ºC refrigeration and stored at 25ºC for up to 7 days, at 30ºC for up to 24 hours and at 32ºC for up to 12 hours. Vaccine was then reconstituted in 100 mL water at defined intervals and potency via colony forming unit (CFU) assay was performed. To assess the stability of reconstituted vaccine, buffer and vaccine packets were reconstituted with 100 mL of water, left at room temperature and potency was measured at defined 0.5, 1, 2 and 4 hours. Finally, long-term potency was measured after vaccine and buffer packets were removed from 2-8ºC refrigeration, cycled up to 3 times at 25ºC for a total of 24 hours, and stored again at 2-8ºC. Potency is defined as 4×10^8 to 2×10^9 CFUs per dose. Vaccine/buffer packets maintained potency for up to 5 days at 25°C, for up to 12 hours at 30°C, and for up to 6 hours at 32°C. Following a 24 hour excursion at 25°C, long-term potency at 2-8°C was not affected. Reconstituted PXVX0200 maintained potency for 4 hours at room temperature. In conclusion, PXVX0200 maintains stability under a variety of environmental conditions. This will facilitate self administration.

367

EPIDEMIOLOGY OF VIRAL HEPATITIS AND HEALTH LITERACY IN CULTURALLY AND LINGUISTICALLY DIVERSE COMMUNITIES IN SOUTH EAST QUEENSLAND, AUSTRALIA

Belaynew Wasie Taye[1], Patricia C. Valery[2], Burglind Liddle[3], Aidan Woodward[1], Donata Sackey[3], Paul J. Clark[4]

[1]*University of Queensland, Brisbane, Australia,* [2]*QIMR Berghofer Medical Research Institute, Brisbane, Australia,* [3]*Mater Health, Brisbane, Australia,* [4]*University of Queensland, Greenslopes Clinical Unit and Mater Hospitals, Brisbane, Australia*

Studies investigating the health literacy about viral hepatitis in people from culturally and linguistically diverse (CALD) backgrounds are limited. This study explored the epidemiology of viral hepatitis and health literacy about viral hepatitis and liver disease in a CALD patient group in South East Queensland. Adult patients (n=66) of CALD background diagnosed with HBV or HCV participated in the study. Clinical and laboratory data were captured and encoded into the REDCap database. Health literacy was assessed using a 5-question, 12-point scale. Variance weighted linear regression was used to identify factors associated with knowledge about viral hepatitis. The median age of patients was 40.2 years (interquartile range (IQR) 32.8-47.6), and the majority (83.3%) were overseas-born. Three-quarters of patients were diagnosed with HBV. The most prevalent HCV genotype (G) was G3 (43.8%) and all patients with HCV G4 (12.5%) were born outside of Australia. At least advanced liver fibrosis (transient elastography score ≥10kpa) was reported in 20.8% of patients. The median knowledge score was 7.8 (IQR 6-9) on a 12-point scale. The strongest predictors of knowledge of viral hepatitis were patient's educational level (secondary, β=4.8, $p<0.0001$ or tertiary, β=8.1, $p<0.0001$), transition through a refugee camp (β= -1.2, p=0.028), and country of diagnosis (β=-1.9, p=0.016). In conclusion, migration continues to shape the epidemiology of viral hepatitis in Australia. There is a room for improvement of knowledge of and reduce misconceptions about viral hepatitis transmission in CALD communities.

368

COMPARISON BETWEEN SD BIOLINE DENGUE DUO TEST AND INBIOS' PROTOTYPE DENGUE IMMUNOCHROMATOGRAPHIC TESTS

Hua-Wei Chen[1], Tania Maldonado[1], Christina Farris[1], Victor Sugiharto[1], Mark Simons[1], Gabriel Defang[1], Calli Rooney[2], Shuenn-Jue Wu[1]

[1]*Naval Medical Research Center, Silver Spring, MD, United States,* [2]*US Army Medical Materiel Development Activity, Fort Detrick, MD, United States*

Dengue virus (DENV) transmission is endemic in more than 120 countries with an estimated 400 million infections occurring annually. Currently, there is no effective vaccine or specific antiviral therapeutic to prevent or treat dengue for U.S. military personnel. Accurate diagnosis followed by attentive supportive care to manage severe dengue can improve the outcome of patients. PCR based and ELISA based assays are available for dengue diagnosis. However, these diagnostic assays usually require trained personnel and specialized equipment which are not

suitable for routine work, especially in resource-constrained areas. Two Immunochromatographic Test (ICT) prototype devices from InBios (Seattle, WA) and an Abbott/Alere SD Bioline Dengue Duo test (Chicago, IL) were compared in this study. Total of 130 well-characterized de-identified clinical samples were selected, including 25 acute-convalescent pairs of DENV3, 20 pairs of DENV4, 10 non-DENV febrile samples and 10 normal human sera from Naval Medical Research Unit Two along with 20 Zika clinical samples (5 antigen positive and 15 antibody positive) from the Naval Infectious Diseases Diagnostic Laboratory. Assays were performed according to the manufactures' instructions. Our results showed that the InBios Traditional cassettes had better overall performance than the Multiplex cassettes when compared to the SD Duo test as the reference test (94.8% sensitivity and 87.9% specificity to 90.9% sensitivity and 51.5% specificity, respectively). Both cassettes had identical sensitivity (96.0%) and specificity (96.5%) on NS1 detection. Traditional cassettes performed better on IgG detection than the Multiplex cassettes. For different serotypes, the Traditional cassettes had 93% overall sensitivity for DENV3 and 100% overall sensitivity for DENV4. All three dengue rapid tests cross-reacted with Zika clinical samples. In conclusion, the SD Bioline Duo test and the InBios Traditional format have similar overall performance and are better than the InBios Multiplex format.

369

WHAT IS THE IMPACT OF MASS OR SYSTEMATIC ANTIBIOTIC ADMINISTRATION ON ANTIBIOTIC RESISTANCE IN LOW-MIDDLE-INCOME COUNTRIES? A SYSTEMATIC REVIEW

Lison Rambliere[1], Didier Guillemot[1], Elisabeth Delarocque-Astagneau[2], Bich-Tram Huynh[1]

[1]*Institut Pasteur/Inserm /Université de Versailles Saint Quentin (UVSQ), Paris, France,* [2]*Inserm/Université de Versailles Saint Quentin (UVSQ)/ Assistance publique – Hôpitaux de Paris (APHP), Paris, France*

Antibiotic consumption is a key driver of antibiotic resistance (AR), particularly in low- and middle-income countries (LMICs) where risk factors for its emergence and spread are rife. Mass or systematic drug administration (MDA/SDA) of antibiotics is increasingly used to control the spread of particular infectious disease or to prevent bacterial infections in at risk patients. Both of these repeated individual and/or large population exposures to antibiotics may play a critical role in the emergence and spread of AR. However, their potential contribution is unknown.We conducted a systematic review to provide an overview of all MDA/SDA in LMICs that can potentially target a substantial part of the population, indications, antibiotics used and, if investigated, impact on AR.Of the 2193 articles identified through our search 67 were reviewed. Indications were various and targeted populations were particularly vulnerable. Overall, the most commonly used antibiotic were co-trimoxazole in HIV-infected people to reduce the risk of infections, amoxicillin to improve infant weight gain and azithromycin to prevent infant and newborn morbi-mortality and delivery complications.AR was evaluated in 14 studies (40%) of which 10 used a longitudinal design, 11 phenotypic and/or 4 genomic invetigation. Although the proportion of *Escherichia coli* (>50%) and *Streptococcus pneumoniae* (>75%) resistant to co-trimoxazole were already high at baseline in the majority of co-trimoxazole SDA setting. Three studies reported an increase of resistance in these pathogens in groups receiving antibiotics compared to the control. Following azithromycin MDA/SDA, increase in macrolide resistance, both in metagenomic and phenotypic analysis (*S. pneumoniae* and *Staphylococcus aureus*) were reported. We showed that a substantial proportion of the population may already receive MDA/SDA in LMICs. There is already some evidence of impact on AR although less than half of the studies reported AR data. Evaluation of potential consequence on AR prior to MDA/SDA implementation is needed, combined with standardized AR surveillance for timely detection of AR emergence.

370

THE IMPACT OF LEPROSY REACTIONS: A QUALITATIVE AND PARTICIPATORY STUDY IN INDONESIA AND INDIA

Ruth Peters[1], Annisa Ika Putri[2], Kevin De Sabbata[1], Barbara de Barros[3], Regitta Indira Agusni[4], Joydeepa Darlong[5], Stephen Walker[3]

[1]*Vrije Universiteit Amsterdam, Amsterdam, Netherlands,* [2]*Airlangga University, Surabaya, Indonesia,* [3]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [4]*Ailangga University, Surabaya, Indonesia,* [5]*Leprosy Mission Trust, New Delhi, India*

Leprosy reactions are immune-mediated complications of leprosy which play a significant role in the morbidity of people affected by the disease. While a considerable amount of literature has been published on the social, practical, and relational impact of leprosy in general, still very few studies focus specifically on the experiences of people with leprosy reactions. This hampers the effective management and treatment of the condition. The main objective of this study is to investigate the impact of leprosy reactions on the life of people affected by analysing their life experiences and their perspectives about the condition in two endemic countries: India and Indonesia. In doing this, it uses a participatory approach. The main study population are patients that experience type 1 or type 2 reactions at Soetomo General Hospital, Indonesia or Purulia Leprosy Mission Hospital, India. We used purposive sampling for the selection of our sample. In-depth interviews with in total 66 participants were held. Content and conversational analysis were done. The results show that leprosy reactions are perceived as an unpredictable and painful condition. Respondents reported delays in the diagnosis and difficulties with activities such as going to the bathroom, sleeping, eating, cooking and studying. In the interviews, participants expressed a whole range of emotions and feelings including confusion, sadness, anxiety and anger. Reasons included the slow progress and concerns related to the medicine. The leprosy reactions had a major impact on their social life, financial situation and several participants told us they felt stigmatized. Differences between the two settings were identified and include how participants cope with the reactions (e.g., staying at home and disguising the name of leprosy versus disclosure and continue working until hospitalization). Participants indicated the need for (peer) support. We recommend testing of interventions such as peer counselling, participatory video and teleconsultation.

371

LITTLE DROPS MAKE AN OCEAN: HOW COMMUNITY-BASED HEALTH INSURANCE DOES AN OCEAN OF GOOD AT THE BWINDI COMMUNITY HOSPITAL, UGANDA

Benjamin Norton[1], Scott Kellermann[2], Michael C. Borecky[3], Thomas E. Borecky[3], Birungi Mutahunga[4], Nahabwe Haven[4], Latha Rajan[1]

[1]*Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States,* [2]*Fulbright Scholarship Program, Kanungu, Uganda,* [3]*Loma Linda University School of Medicine, Loma Linda, CA, United States,* [4]*Church of Uganda Bwindi Community Hospital, Kinkizi Diocese, Kanungu, Uganda*

The Bwindi Community Hospital (BCH) is a community-based hospital in Kanungu, Uganda, serving some of the poorest and most remote populations in Uganda. Families subsist on less than $1.90/day and some, such as the indigenous Batwa pygmies, survive on <$0.80 / day. Responding to the acute healthcare needs of these isolated and impoverished people, a microinsurance program – eQuality Health Insurance (eQHI) – was initiated as a pilot. Although it was anecdotally a success, there was a pressing need to evaluate program impact. Neonatal mortality is now the biggest issue in under-five mortality. Studies in 2000 indicated 18% neonatal mortality and 38% under-five mortality among Batwa children. Hence, this study was undertaken to assess the impact of eQHI on birth and neonatal outcomes in patients seen at BCH. We performed a four-year case-controlled retrospective chart review of birth

and neonatal outcomes. For each year, all stillbirths were included; live births were randomly selected from all eligible live births at a 4:1 ratio to stillbirths. All neonatal deaths were included, and surviving neonate controls were randomly selected at a 4:1 ratio to neonatal deaths. In the live vs. stillbirth study, 155 stillbirths were compared with 760 live birth controls. Having insurance was significantly associated with a higher chance of live birth (87.1% vs 64.9%; RR 1.34 (CI 95%: 1.28-1.40); OR 3.64 (CI 95%: 3.44-3.84); p<0.0001). Age-related risk factors (women <20 or >35 years old) were not statistically significant (p>0.05). In the neonatal survival study, 86 neonatal deaths were compared with 360 surviving infant controls. Having insurance was associated with higher neonatal survival (85.7% vs 77.6%; RR 1.11 (CI 95%: 1.05-1.15); OR 1.77 (CI 95%: 1.47-2.00; p<0.05). Being a retrospective study, a limitation was that risk factors for stillbirth and poor neonatal outcomes were not recorded. We conclude that a novel microinsurance scheme introduced in the BCH catchment area resulted in markedly reducing stillbirths and neonatal mortality. Further steps are being taken to expand coverage to most, if not all, of this severely disadvantaged population.

372

THERAPEUTIC POTENTIAL OF *WITHANIA SOMNIFERA* IN FILARIAL INDUCED SECONDARY LYMPHEDEMA

Anand Setty Balakrishnan

Madurai Kamaraj University, Madurai, India

Lymphatic filariasis is a major parasitic disease caused by nematodes *Brugia malayi* and *Wuchereria bancrofti*. Also in this secondary lymphedema, the natural ability of lymphatic vessels to form new lymphatic channels is yet to be studied. Our present study aimed at evaluating the anti-bacterial property of *Withania somnifera* (ashwagandha), a highly revered ayurvedic plant in India, against *Bacillus cereus* and *Staphylococcus epidermis* associated with lymphedema. Our studies also examined the lymphangiogenic potential of ashwagandha in response to adult *Brugia* worm homogenates in human dermal lymphatic endothelial cells (HDLECs). The purified powder of ashwagandha is well characterized analytically. Ashwagandha shows a clear zone of inhibition in both paper disc and agar diffusion methods, indicating significant anti-bacterial activity against the two bacterial strains. *In-vitro* lymphangiogenic activity of ashwagandha was evaluated by 2D matrigel using HDLECs in response to whole *Brugia* worm homogenates (50ng/ml). These studies showed that adult worm homogenate attenuated the tubular network formation. Interestingly addition of ashwagandha restored the endothelial tube formation in a dose dependent manner. To further dissect out the process of bacterial killing and internalization by host cells, we employed HDLEC culture and chemical inhibition approach by blocking the cellular uptake system. Bacterial Infection Assay and Viable cell count analysis were carried out to evaluate the internalization of bacteria into the host cells and the viability of host cells after infection respectively. We will be further performing RT-PCR analysis to confirm the infection-induced gene expression and Confocal Imaging to check the infection-induced morphological changes and cytotoxicity in host cells. Taken together, our results shows ashwagandha efficiently kills bacteria, augments lymphangiogenesis and may supports reduction of swelling in lymphedema subjects. Thus ashwagandha shown to be a novel therapeutic agent in the treatment of filarial induced secondary lymphedema.

373

IS CROSS REACTIVITY OF ALERE FILARIASIS TEST STRIP IN PERSONS WITH LOIASIS ASSOCIATED TO RELEASE AND CLEARANCE CYCLES OF *L. LOA* ANTIGENS?

Linda Djune Yemeli[1], Marla Hertz[2], Hugues Clotaire Nana-Djeunga[1], Jean Gabin Bopda[1], Joseph Kamgno[1], Philip Budge[3]

[1]*Centre for Research on Filariasis and other Tropical Diseases, Yaounde, Cameroon,* [2]*Department of Medicine and Infectious Diseases Division, Washington University in St. Louis, St Louis, MO, United States,* [3]*Department of Medicine and Infectious Diseases Division, Washington University, St Louis, MO, United States*

Cross reactivity of lymphatic filariasis rapid diagnostic tests (LF-RDTs) with loiasis is an obstacle to LF elimination in areas of co-endemicity. High *L. loa* microfilaria (Mf) loads are correlated with false-positive LF-RDTs, but not all individuals with high *L. loa* Mf loads test positive, and some heavily infected persons revert to negative status despite maintaining high Mf loads. This suggests an intermittent release and/or clearance of cross reactive *L. loa* antigens (CRLAgs). We have initiated a prospective cohort study in Okola (Centre Region, Cameroon) to investigate how, why, and when CRLAgs are released and cleared. The study area is endemic to onchocerciasis, loiasis and *Mansonella perstans*, but not to LF. We recruited adult volunteers previously excluded from the onchocerciasis test-and-not treat (TaNT) program due to *L. loa* Mf loads >20,000/mL. Of 135 individuals screened, 70 (52%) tested positive by Filariasis Test Strip (FTS). Daytime *L. loa* Mf counts were higher among those testing FTS positive (median 34,450; range 6,680-114,240) than among those testing FTS negative (median 10,180; range 0-98,120). All individuals testing FTS-positive and 13 FTS-negative controls with *L. loa* Mf counts >20,000 (range 21,780-98,120) were enrolled into the prospective study. Enrollees are aged 21 to 80 years and 65% are male. No enrollees had evidence of LF by nocturnal thick blood smear; 7.2% tested positive for intestinal helminths (by Kato-Katz), and 6% are co-infected with *Mansonella perstans*. Identification of specific circulating CRLAgs by mass spectrometry is pending. Prospective quarterly monitoring of this cohort will help decipher whether specific *L. loa* Ag are reliably present and will inform on the nature of these Ag.

374

WHITE BLOOD CELL DIFFERENTIALS, CYTOKINES, TOTAL IGE AND HISTAMINE DISTINCTION BETWEEN IVERMECTIN-TREATED AND UNTREATED *ONCHOCERCA*-INFECTED PATIENTS IN THE NKWANTA NORTH DISTRICT, GHANA

Joshua Labadah[1], Kwadwo A. Kusi[2], Michael D. Wilson[3]

[1]*West African Centre for Cell Biology of Infectious Pathogens, University of Ghana/(University of Health and Allied Science, Ho), Accra, Ghana,* [2]*Noguchi Memorial Institute of Medical Research, University of Ghana, Accra, Ghana,* [3]*Noguchi Memorial Institute for Medical Research, University of Ghanaiversity of Ghana, Accra, Ghana*

River blindness (onchocerciasis) is a neglected tropical disease caused by the nematode *Onchocerca volvulus*. It mainly affects the eye and the skin. The microfilariae stage of the parasite is known to be responsible for the pathology of the disease. Ivermectin (IVM), the recommended treatment drug is effective against the microfilariae (killing about 60% within 24 hours and 99% within 7 days) and thereby resolving the symptoms. IVM also suppresses the adult female parasite's ability to produce new microfilariae at least within 90 days. This study sought to identify some immune factors that distinguish between ivermectin-treated and untreated microfilaremic individuals to serve as markers for complementary diagnosis and treatment efficacy, and also help in understanding the disease mechanism. White blood cell differentials were measured using automated hematology analyzer for 124 adults; 53 microfilaremic (MF) and 71 ivermectin-treated amicrofilaremic (AMF) individuals 3 months following last treatment at the Nkwanta North District of Ghana. Sub groups ($17 \leq n \leq 20$) of both MF and first time IVM-treated AMF were assessed for plasma cytokines using Luminex multiplex bead assay, total IgE and urine histamine by Enzyme-linked immunosorbent assay. Comparisons were made by Mann-Whitney U tests at 0.05 alpha. The geometric mean microfilariae density/milligram skin snip was 22.5 (range 5.8-56.3). Basophil count was lower (p=0.0001) while eosinophil count was higher (p=0.02) for MF compared to AMF. Neutrophils, lymphocytes and monocytes were not significantly different between the two groups. Out of 14 cytokines, Interleukin (IL)-13 (p=0.002), IL-8 (p=0.03) and Interferon-gamma (p=0.003) were higher among MF compared to first time treated AMF. Histamine and IgE were not different between the two groups.

IL-13, IL-8 and interferon-gamma, basophils and eosinophils distinguished the two groups and this knowledge could be helpful in the diagnosis, treatment, understanding the disease and support efforts towards its elimination.

375

EVALUATING THE USE OF OV-16, *OV*-CPI-2 AND *OV*-B20 AS SEROLOGICAL BIOMARKERS FOR EARLY DETECTION OF *ONCHOCERCA OCHENGI* INFECTION IN NATURALLY INFECTED CAMEROONIAN CALVES

Gemma Farrington[1], **Lisa Luu**[1], Catherine Hartley[1], Germanus Soh Bah[2], Vincent N. Tanya[3], David Abraham[4], Thomas Nutman[5], Bin Zhan[6], Sara Lustigman[7], Benjamin Makepeace[1]

[1]*University of Liverpool, Liverpool, United Kingdom,* [2]*L'Institut de Recherche Agricole Pour le Développement (IRAD), Yaounde, Cameroon,* [3]*L'Institut de Recherche Agricole Pour le Développement (IRAD),, Yaounde, Cameroon,* [4]*Thomas Jefferson University, Philadelphia, PA, United States,* [5]*National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States,* [6]*Baylor College of Medicine, Houston, TX, United States,* [7]*New York Blood Centre, New York, NY, United States*

Human onchocerciasis affects 20 million people, mostly in sub-Saharan Africa, leading to severe dermatitis and visual impairment. Diagnosis of the disease can be performed by either skin snip microscopy and/or PCR, or by serodiagnosis using *Ov*-16 as the target antigen. However, diagnostic sensitivity may be poor in hypoendemic areas of infection. There is also a need for diagnosis of pre-patent infection, such as antibody recognition of *Onchocerca*-specific larval antigens, to identify and potentially treat infections before the adult worms mature. In previous studies using experimental infections of chimpanzees and calves, some larvae-specific antigens were tested and shown to be potentially informative. Here, we evaluate whether the antigens *Ov*-16 (larvae/adult), *Ov*-CPI-2 (larvae/adult) and *Ov*-B20 (larvae) can be used as onchocerciasis biomarkers in calves naturally exposed to ongoing transmission. Seroconversion to these larval antigens may identify a biomarker that can detect early-stage infections even in the absence of adult worms and patent infection. Using an ELISA-based approach, we evaluated the detection of total IgG responses against these antigens in sera collected from untreated calves (control group; n = 10) or calves treated prophylactically with ivermectin (IVM; 150 µg/kg every three months; n = 10). Sera were sampled every 4 weeks over one year. *Ov*-B20 responses were found to be high prior to exposure, suggesting cross-reactivity with other parasitic nematodes, and no differences in reactivity were observed between control and IVM-treated calves. In contrast, *Ov*-16 responses were low at baseline, then increased marginally in the IVM-treated group. However, they climbed significantly in control animals before plateauing by 28 weeks, suggesting that peak *Ov*-16 responses occur after the final larval moult. *Ov*-CPI-2 responses remain under evaluation and will be presented. In conclusion, the cattle model presents an ideal system to test not only the pathogen- and stage-specificity of antigen recognition but also the impact of chemotherapy on the serological response to *Ov*-16 and other *Onchocerca* antigens.

376

DEVELOPMENT OF NOVEL SEROLOGICAL ASSAYS FOR NEGLECTED TROPICAL DISEASES: NEED FOR WELL CHARACTERIZED SAMPLE PANELS

Vitaliano A. Cama[1], Guilherme Maerschner Ogawa[1], Ole Lagatie[2], Maan Zrein[3], Lieven Stuyver[2]

[1]*CDC, Atlanta, GA, United States,* [2]*Janssen Global Public Health, Beerse, Belgium,* [3]*InfYnity Biomarkers, Lyon, France*

Onchocerciasis assessments use an IgG4 OV-16 ELISA, with reported high specificity (>99%) but low sensitivity (45-88%), suggesting improved assays are needed. Previously, 8 onchocerciasis serology candidates were identified through multi-nomic approaches. CDC tested the performance of the peptides with a characterized serum panel, and further evaluated the best diagnostic candidates to determine time of seroconversion. The 8 peptides were tested using 100 characterized samples: 50 from onchocerciasis-infected, seropositive people, and 50 from people from non-endemic areas (controls). The anti-*Onchocerca* sensitivity and specificity for the 8 peptides was between 73-88%, and 77-100%, respectively. The best 4 peptides were further tested to determine time to seroconversion with 372 samples from primates or humans with and without onchocerciasis or other filarial infections: 292 samples from non-human primates (NHP) laboratory-infected with onchocerciasis (collected monthly for 5-years), and 80 from humans without onchocerciasis (40 OV-16 negative, 14 positive to either *Loa loa* or *Mansonella perstans*, 26 OV-16 negative from formerly endemic areas). Peptides were tested using an IgG ELISA-based multiplex micro-assay, where peptides, OV-16 and internal controls were dot-printed in triplicate per well in 96-well plates. Antibody reactivity was quantified by image analysis of the microdots. Panels from each *Onchocerca*-inoculated NHP had well defined seroconversion time points against OV-16, without evidence of sero-reversion . However, NHP panels did not react against any of the 4 peptides. Data from the 80 negative samples showed negative reactivity against OV-16 but high background reactivity against the novel peptides. These findings support the utility of multiplex micro-assay platform for simultaneous analyte evaluation, allowing efficient use of reagents, labor, and samples of limited availability. Although the novel peptides were not optimal for onchocerciasis serology, the study demonstrates the importance of well-characterized serum panels in developing novel serological assays for onchocerciasis.

377

EMODEPSIDE AS MACROFILARICIDE FOR THE TREATMENT OF RIVER BLINDNESS: AN INTERDISCIPLINARY AND COLLABORATIVE APPROACH TOWARDS A MEANINGFUL PROOF OF CONCEPT FOR THE PRECLINICAL PACKAGE

Helena Fieseler[1], Ralph Krebber[2], Sebastian Schneckener[3], Kristine Fraatz[1], Germanus S. Bah[4], Simon Townson[5], Benjamin L. Makepeace[6], Gabriele Schmuck[1], Martin Glenschek-Sieberth[7], **Daniel Kulke**[1]

[1]*Bayer Animal Health GmbH, Leverkusen, Germany,* [2]*Bayer AG - Division Crop Science, Leverkusen, Germany,* [3]*Bayer AG, Leverkusen, Germany,* [4]*Institut de Recherché Agricole pour le Développement, Bamenda, Cameroon,* [5]*Northwick Park Institute for Medical Research, Harrow, United Kingdom,* [6]*University of Liverpool, Liverpool, United Kingdom,* [7]*Bayer AG - Division Pharmaceuticals, Leverkusen, Germany*

The current strategy to control onchocerciasis (river blindness) relies on mass drug administration using ivermectin, which shows robust efficacy against *Onchocerca volvulus* microfilariae but has little impact on adult worm lifespan. Thus, a macrofilaricidal drug is urgently needed to effectively cure patients and sustainably disrupt the parasite's lifecycle. The anthelmintic drug emodepside is currently being developed by Drugs for Neglected Diseases initiative and Bayer AG as a macrofilaricide against *O. volvulus*. As part of the preclinical package, the efficacy of emodepside was evaluated in the closely related cattle parasite *Onchocerca ochengi*, which is not established in a laboratory setting in its natural host. Drug evaluation thus requires prolonged field studies with naturally infected cattle in sub-Saharan Africa. Therefore, this collaboration required a clear rationale and robust study protocol in order to generate reliable data compatible with good laboratory practice. Our path towards the in-life phase of the main cattle study, including the decisions on route of administration, the service formulation and the positive control is described. We performed an ascending dose study in Holstein cattle in Germany with experts from Bayer Animal Health and L'Institut de Recherche Agricole pour le Développement, Cameroon. An analytical method to measure emodepside in skin and nodules was established, followed by modelling of single and multiple humanized doses for the main cattle study. The determined maximum tolerable dose for Holstein cattle was confirmed for zebu (Ngaoundéré Gudali breed) at the study site in Cameroon. Following completion of the draft (including decisions on study groups, number of animals, sample collection, study duration etc.), the study protocol was reviewed by an external scientific advisory

board. In summary, by forming an interdisciplinary and highly dedicated team, a collaboratively designed protocol for the cattle study ensured an informative outcome. The study clearly supports the development of emodepside for the treatment of human onchocerciasis.

378

MODEL BASED CHARACTERIZATION OF THE PHARMACODYNAMIC EFFECT OF EMODEPSIDE ON *ONCHOCERCA OCHENGI* IN AFRICAN CATTLE

Sebastian Schneckener[1], Benjamin L. Makepeace[2], **Daniel Kulke**[3], Martin Glenschek-Sieberth[4]

[1]*Bayer AG, Leverkusen, Germany,* [2]*University of Liverpool, Liverpool, United Kingdom,* [3]*Bayer Animal Health GmbH, Leverkusen, Germany,* [4]*Bayer AG - Division Pharmaceuticals, Leverkusen, Germany*

The current strategy to control onchocerciasis relies on mass drug administration using ivermectin, which shows robust efficacy against microfilariae but has little impact on adult worm lifespan. Thus, a macrofilaricidal drug is urgently needed to effectively cure patients and sustainably disrupt the parasite's lifecycle. Emodepside is currently being developed by DNDi and Bayer AG as a macrofilaricide against *O. volvulus*. As part of the preclinical package, the efficacy of emodepside was evaluated in the closely related cattle parasite *Onchocerca ochengi*. Here, PKPD modelling was used to establish an effect of emodepside on *O. ochengi*. In a field study, naturally infected animals were randomised into six treatment groups (n = 7 animals per group): Melarsomine (positive control) or vehicle only (negative control) alongside emodepside as a single or multiple dose at two dose levels. This work quantifies the effect of emodepside on *O. ochengi* microfilaria (Mf) counts in the skin, and the adult female and male worm motility. We tested the hypothesis that emodepside has a dose-dependent effect on these endpoints. Mf density in skin was assessed over a time period of 550 days. As a robust approach to aggregate individual observations, we calculated the integral of the curve that describes Mf density over time, the Mf load. Increasing total doses of emodepside lead to a significant ($p = 0.004$) decrease in Mf load. Male and female worm motility was observed over 550 study days and a linear model was used to describe a possible decrease in motility per treatment group. We found that a) female motility significantly ($p < 0.05$) decreased in all treatment groups, excluding placebo; b) the decrease in motility of male worms was observable, but not significant except for the highest dose level of emodepside, while the dose-dependent effect on male motility in general was a significant observation ($p < 0.05$). In summary, we were able to demonstrate the significant pharmacological activity of emodepside in cattle infected with *O. ochengi,* confirming emodepside as candidate for clinical development to treat human onchocerciasis with potential to kill adult worms.

380

ONCHOCERCIASIS ELIMINATION IN SENEGAL: STATUS AND POTENTIAL CROSS BORDER CHALLENGES

Ngayo Sy[1], Fatou Ndiaye Badiane[1], Amadou Doucoure[1], Daniel Boakye[2], Charles Mackenzie[2], Jamie Tallant[2], Sara Laskowski[2], Sashi Leff[2]

[1]*Ministry of Health and Social Action, Dakar, Senegal,* [2]*END Fund, New York, NY, United States*

Onchocerciasis is endemic in 8 districts in three regions of Senegal, with baseline *Onchocerca volvulus* infection prevalences (skin skip) ranging from 19 - 82%. These endemic areas fall within two river basins considered as transmission zones for evaluation purposes; the Faleme and Gambia. Control interventions (vector control, mass drug administration with ivermectin) have been undertaken since 1987 through the OCP, APOC and other partners. Epidemiological and entomological evaluations undertaken between 2006 and 2011 indicated that significant progress has been made and that interruption of transmission may have been achieved. Following the change in the programme paradigm from control to elimination, entomological assessments completed in 2015 for the two basins were all below the threshold of 1 infective fly per 2000 vector *Simulium damnosum* flies: (0.05%) - Faleme (0.04) and Gambia (0.04). In 2017, the Senegal Onchocerciasis Elimination Expert Committee recommended that epidemiological and entomological evaluations should be carried out as per 2016 WHO guidelines for the verification of onchocerciasis elimination. In 2018, *S. damnosum* s.l. vectors were collected at 7 and 10 sites in the Faleme and Gambia basins respectively. After pool-screening PCR of a total of 47,371 and 55,508 flies from the two basins, the upper 95% limit of vector infectivity rates were seen to be 0.04% and 0.05%, supporting the contention that transmission in these basins meets the criteria for interruption of transmission, pending OV16 serological results. However, two nearby cross-border sites, one in Mali and one in Guinea are known to have higher infectivity rates - (Mousala, 2.0%) and 0.3% (Thiocoye Pont, 0.3%) respectively; samples from 2019 entomological assessments and Ov16 serology are currently being processed. Data to date indicates the important global milestone that Senegal may be among the first countries where interruption of onchocerciasis transmission has been achieved nationwide. Such an important conclusion will necessitate active monitoring of cross-border sites that could pose a challenge for recrudescence.

381

STRATEGIES TOWARD MEETING THE LYMPHATIC FILARIASIS ELIMINATION GOAL IN TANZANIA

Denis Kailembo[1], Gerald Robi[1], Veronica Kabona[1], Cecilia Uisso[2], Alistidia Simon[2], Oscar Kaitaba[2], Faraja Lyamuya[3], Kerry Dobies[4], Mary Linehan[4], Abdel Direny[4], Josh West[5], Benjamin Crookston[5]

[1]*IMA World Health, Dar Es Salaam, United Republic of Tanzania,* [2]*Ministry of Health, Community Development, Gender, Elderly and Children, Dar Es Salaam, United Republic of Tanzania,* [3]*Ministry of Health, Community Development, Gender, Elderly and Children, Dodoma, United Republic of Tanzania,* [4]*IMA World Health, Washington, DC, United States,* [5]*Bringham Young University, Provo, UT, United States*

Lymphatic Filariasis (LF) is endemic across 120 districts in Tanzania. Tanzania's Neglected Tropical Diseases Control Program (TZNTDCP) is using the drug-package of Ivermectin+Albendazole (IVM+ALB) during mass drug administration (MDA) as the primary strategy for achieving LF elimination. Among the 120 LF endemic districts, 105 districts have passed transmission assessment survey (TAS), reaching the criteria to stop MDA. While the TZNTDCP continues surveillance in districts which passed TAS, the focus is now on the remaining 15 districts with persistent LF transmission. TZNTDCP added extra clusters that were positive in TAS1 and TAS2 during TAS2 and TAS3 conducted in 2019. No positive cases were reported in the extra clusters. Of the 15 districts still requiring MDA, 14 were scheduled for re-pre-TAS in 2020 (to determine eligibility for conducting TAS) after successfully achieving sufficient coverage in the last two rounds of MDA. During this re-pre-TAS, three to five sites within each district were selected. The sites included both sentinel and spot check sites from previous assessments with a history of failure (Ag >2%), as well as new sites selected from those with reported cases of LF and a history of low MDA coverage. About 300 individuals aged 5 years and older were tested per site with Filarial Test Kits (FTS). Preliminary results showed that 8 of the 14 districts did not pass re-pre-TAS, highlighting ongoing transmission. TZNTDCP will develop and implement new strategies to strengthen MDA and to reach all populations (including migrant populations) before the next disease specific assessment (DSA). The strategies include allocation of community drug distributors (CDDs) according to level of effort (applying the CDD ratio), implement micro-plans at the sub-district level, and enhance supportive supervision. Moreover, DSA failure investigation will be conducted to critically analyze and provide recommendations to address why the districts did not pass the assessment. These strategies will help TZNTDCP move towards LF elimination in line with the WHO proposed target of eliminating LF as a public health problem by 2030.

382

INTEGRATING COMMUNITY HEALTH WORKERS IN MASS DRUG ADMINISTRATION (MDA) SUPERVISION TO IMPROVE MDA IMPLEMENTATION

Eurica Denis[1], Alain Javel[1], Carl Renand Fayette[1], Paul-Emile Dalexis[1], Marc-Aurele Telfort[2], Ellen Knowles[3], Mary Linehan[3], Abdel Direny[3], Josh West[4], Benjamin Crookston[4]

[1]*IMA World Health, Port au Prince, Haiti,* [2]*Ministry of Health and Population, Port au Prince, Haiti,* [3]*IMA World Health, Washington, DC, United States,* [4]*Brigham Young University, Provo, UT, United States*

To achieve global goals to eliminate lymphatic filariasis (LF), the Haiti NTD Control Program (HNTDCP) follows WHO's recommendations to implement rounds of annual mass drug administration (MDA) of diethylcarbamazine and albendazole for at least five consecutive years among at risk populations to halt LF transmission. In Haiti, MDA has always been supervised by the Ministry of Health (MOH) staff and its partners. The MDA strategy employs school- and community-based distribution posts to distribute the drugs to the targeted population. Due to a lack of resources and accessibility, some distribution posts have not been adequately supervised during MDA, resulting in poor reporting and low epidemiological coverage (<65%) by community drug distributors (CDDs). In 2019, the Haiti Neglected Tropical Disease Control Program (HNTDCP) developed a multi-level supervision plan to improve MDA supervision. The first level included a platform of 157 community health workers (CHWs) in 12 communes to ensure each distribution post is visited daily. The second level of supervision was comprised of MOH representatives and implementing partners, providing technical and logistical support to the first level supervisors, or directly to the distribution posts. With this new strategy, each distribution post is supervised every day using an electronic supervision checklist. The electronic forms are completed by each CHW using Open Data Kit through mobile phone, in which data is sent to an online server. A total of 1400 out of 1501 distribution posts (93%) are now supervised daily and geo-localized. Geo-localization is important to avoid uncovered areas during MDA. In addition, partial MDA results from every distribution post is collected daily and other information such as cleanliness of the distribution post and directly observed treatment strategy were also reported. With the information collected, HNTDCP was able to evaluate coverage daily and provide necessary assistance where needed, as well as inform program adaption in real-time to ensure effective coverage was reached. MDA supervision and reporting quality have improved while maintaining low costs.

383

THE IMPLEMENTATION OF A PRE-STOP MASS DRUG ADMINISTRATION FOR THE 'LAST MILE' OF ONCHOCERCIASIS ELIMINATION IN MALI AND CHAD

Daniel Boakye[1], Jamie Tallant[1], Moussa Sow[2], Lokemla Nadjilar[3], Sara Laskowski[1], Sashi Leff[1]

[1]*END Fund, New York, NY, United States,* [2]*Ministry of Health, Bamako, Mali,* [3]*Ministry of Health, N'djamena, Chad*

The African Programme for Onchocerciasis Control 2010 conceptual framework (CF) for the elimination of onchocerciasis recommended that the decision to stop treatment with ivermectin be in two steps: Phase 1a - an epidemiological survey, and Phase 1b - both epidemiological and entomological evaluations. Phase 1a requires some 10 communities of highest risk to be assessed to demonstrate a decline in infection level towards interruption of transmission before embarking on the more extensive Phase 1b evaluation for the all-important decision to stop treatment. The 2016 WHO guidelines for the verification of onchocerciasis elimination however recommended a Stop-MDA of both epidemiological (OV16 serology) and entomological assessments (O-150 Poolscreening PCR) to determine interruption of transmission before MDA with ivermectin can be stopped without a prior smaller scale evaluation similar to a Phase 1a evaluation. The WHO Onchocerciasis Technical Advisory Sub-committee (OTS), set up in 2017 to provide guidance on the implementation of the 2016 WHO guidelines proposed that only an OV16 serology survey ("Pre-Stop MDA evaluation") similar to the Phase 1a was necessary, however they advised the use of a different protocol; namely the selection of 100 children aged between 5-9 years in 3-5 first line villages per evaluation unit for testing using a lab-based OV16 RDT. However, the evaluation unit was only loosely defined. With support from the Reaching the Last Mile Fund of the END FUND, a detailed Pre-Stop protocol was developed for implementation in Mali and Chad. We report here how this was implemented in Chad and Mali covering planning, delineation of evaluation areas, selection of *Simulium damnosum* s.l breeding sites, first line villages, study participants and sample collection for OV16 RDT. The protocol was easy to implement, cost effective as it uses river basins instead of districts to determine the number of first line villages to select it can be adapted to different countries irrespective of the administrative divisions.

384

LESSONS LEARNED FROM A 24/7 HOTLINE EXPERIENCE DURING URBAN MASS DRUG ADMINISTRATION (MDA) IN HAITI

Paul-Emile Dalexis[1], Carl Renand Fayette[1], Eurica Denis[1], Alain Javel[1], Marc-Aurele Telfort[2], Ellen Knowles[3], Abdel Direny[3], Mary Linehan[3], Josh West[4], Benjamin Crookston[4]

[1]*IMA World Health, Port au Prince, Haiti,* [2]*Ministry of Health and Population, Port au Prince, Haiti,* [3]*IMA World Health, Washington, DC, United States,* [4]*Bringham Young University, Provo, UT, United States*

Reaching the minimum goal of mass drug administration (MDA) coverage (≥65%) for lymphatic filariasis (LF) in urban setting remains a challenge worldwide. The Haiti Neglected Tropical Disease Control Program (HNTDCP) first achieved this goal in the metropolitan area of Port-au-Prince with the first MDA launching in 2012. However, coverage has declined in the following rounds and by 2017, HNTDCP reported one of the lowest coverage rates since the initiative began, 41%. In 2018, the Ministry of Health and partners developed a new strategy to improve MDA coverage. This strategy included: improving social mobilization, increasing drug access, increasing volunteers' visibility, and increasing knowledge of drugs and reducing fears of adverse effects, including implementing a 24/7 hotline to facilitate communication between the HNTDCP and the target population. The purpose of this abstract is to provide details about the 24/7 hotline. Three medical doctors and one nurse were recruited to field incoming calls and answer questions. The hotline was free of charge and began 2 days prior to the launch of MDA, continued through one post-MDA. It was advertised on radio and television. An average of 40 calls were received per day, totaling 3,457 calls. Nearly half (44%) of the calls originated from the metropolitan area; 32% of the callers inquired about the location of the distribution posts, 5% had questions on adverse events, 14% declared they had participated in the actual MDA, and 60% asked general questions. By the conclusion of the 2018 MDA, the coverage rate reached 80%. While the hotline was just one component of the revised strategy, it alone cannot be attributed to the increase in MDA coverage. Nevertheless, it appears the hotline was an important factor in the revamped MDA strategy. The HNTCP is considering extending the hotline for MDA in other communes to improve coverage in order to reduce persistent LF transmission in areas which have continuously failed disease specific assessments.

385

CRISPR/CAS9-MEDIATED DISRUPTION OF THE ESCRTII GENE VPS36 GREATLY REDUCES THE SECRETION OF EXTRACELLULAR VESICLES AND THE INFECTIVITY OF *LEISHMANIA MAJOR*

George Y. Dong

McGill University, Montréal, QC, Canada

Leishmaniasis is a widespread neglected infectious disease with 0.9-1.6million new cases every year, resulting in up to 40 000 deaths

annually. Its causative agent, the *Leishmania* parasite, is a protozoan parasite carried by sandflies that infects host macrophages to propagate by evolving various virulence factors, the metalloprotease GP63 in particular, to evade the host immune response. Our lab has shown that exosomes, extracellular vesicles that play a key role in cell-cell communication, released by *Leishmania* parasites contain high amounts of GP63 and increase infection severity when co-injected with *Leishmania* parasite in mice footpads. These characteristics together demonstrate the important role of exosomes in *Leishmania*. To further assess the importance of exosomes to *Leishmania* infection, the gene Vps36, an important member of the ESCRTII complex key to exosome production in eukaryotic cells, was disrupted using CRISPR/Cas9 to insert a puromycin resistance gene in the Vps36 exon of *L. major*, a model parasite responsible for cutaneous leishmaniasis. Vps36null parasites are selected for using puromycin and successful insertion was confirmed using PCR and sanger sequencing. Exosomes are collected using ultracentrifugation and analyzed using nanoparticle tracking analysis, transmission electron microscopy, and LC-MS/MS proteomic analysis. Balb/C mice footpads were injected with WT and Vps36null *L. major* parasite alone, as well as parasites supplemented with purified WT *L. major* exosomes, and skin hyperinflammation was monitored over an 8-week period to compare infection severity. NTA results suggest Vps36null *L. major* parasites produce less EVs during growth, after metacyclic differentiation (stationary phase), and after 37C temperature shock. Vps36null *L. major* failed to induce a high level of infection in the susceptible Balb/C mice even when co-injected with WT *L. major* exosomes compared to severe infection caused by WT *L. major*. These results suggest a key role of Vps36 in exosome production by *L. major* and disrupting its activity reduces EV production and severely impacts infectious capability.

386

SYNTHETIZED ORGANIC COMPOUNDS SHOW LEISHMANICIDAL ACTIVITY AGAINST *LEISHMANIA MAJOR*, THE CAUSATIVE AGENT OF HUMAN CUTANEOUS LEISHMANIASIS

Blaise Dondji, Taylor Henne, Linsey Curry, Timothy Beng

Central Washington University, Ellensburg, WA, United States

Leishmaniasis is a group of anthropozoonoses caused by protozoan parasites of the genus *Leishmania*. The disease is transmitted to the vertebrate host by the female phlebotomine sand fly. It is endemic in 88 countries of southern Europe, Central and South America, Africa, the Middle East and the Indian subcontinent where more than 350 million men, women and children are at risk of acquiring the disease. The number of individuals suffering from leishmaniasis is 12 million worldwide, while 0.5 million new cases of visceral form and 1.5 million of cutaneous form are registered annually. The clinical forms range from cutaneous leishmaniasis with facial and body disfigurations to visceral leishmaniasis in which the parasite spreads into the reticuloendothelial system with fatal outcome in the absence of treatment. Although drugs exist for leishmaniasis, they have been reported to be highly toxic with severe side effects. Such undesirable outcomes include kidney failure and electrolyte imbalances. In addition, current drugs are very expensive and treatment with them requires long duration of hospitalization. Consequently, there is an urgent need for new compounds that are active against *Leishmania*, not toxic or less toxic and affordable. In our quest to identify alternative therapeutic tools for the control of leishmaniasis, we have tested synthesized organic compounds for their leishmanicidal activity against *L. major*, a species causing cutaneous leishmaniasis in humans. We are using a colorimetric assay to assess the activity of these compounds using amphotericin B, an antileishmanial drug as our positive control. Test compounds were evaluated at three concentrations (25, 50 and 100 ug/ml) and the control at 100 ug/ml. Our initial experiment recorded four compounds with higher activity than amphotericin B at 100 ug/ml. In addition, we tested more than 15 more compounds and recorded activity. More interesting was our observation of activity linked to chemical structures of the compounds evaluated. Detailed results will be presented.

387

EVALUATION OF ANTI-LEISHMANIA PROPERTIES OF LACTAM ORGANIC MOLECULES AS POTENTIAL THERAPEUTICS AGAINST HUMAN LEISHMANIASIS

Kenlei Gunther, Cameron Smith, Christy Krischano, Taylor Henne, Linsey Curry, Timothy Beng, **Blaise Dondji**

Central Washington University, Ellensburg, WA, United States

Leishmania are protozoan parasites that cause a group of diseases known as leishmaniasis with three major clinical forms (cutaneous, mucocutaneous, visceral). There are six major species causing disease in humans: *Leishmania tropica, L. major, L. mexicana, L. braziliensis, L. donovani, and L. infantum*. *L. major* was used in our studies. It is the causative agent of cutaneous leishmaniasis (CL) and found in sparsely inhabited regions in west and central Africa, the Middle East, and India. All leishmaniases are transmitted through the bite of sand flies. In the case of CL, ulcers appear at the site of the sand fly bite. The severity of ulcers depends on age and other factors. More than 350 million people worldwide are at risk of becoming infected with leishmaniasis. Surprisingly, there is an overlap between leishmaniasis endemic areas and areas of reporting human immunodeficiency virus infections. Thirty-five countries have reported co-infections with both *Leishmania* and HIV. Current anti-*Leishmania* drugs are highly toxic with serious side effects. Consequently, there is a need to develop safer therapeutic methods. In order to address this lack of safe drugs, lactam organic compounds were tested *in vitro* to identify potential anti-*Leishmania* drugs. Assays were carried out to evaluate the activity of tested compounds against *Leishmania* parasites. Compounds were dissolved in dimethyl sulfoxide (DMSO) and tested at a final concentration of 1% DMSO. This was used as negative control and Amphotericin B, a current anti-*Leishmania* drug, as positive control. Amphotericin B and test compounds were evaluated at 100ug/mL. Alamar Blue dye was used to evaluate activity of compounds tested after incubation for 24, 48 and 72 hours. In living cells, the Alamar Blue is reduced from blue to red and wells show high optical density after the spectrophotometer read. Initial analysis of our data shows activity in several compounds. Additional candidates are being evaluated. In addition, plans are underway to evaluate the toxicity of active compounds against mammalian cells by flow cytometry.

388

RECOMBINASE POLYMERASE AMPLIFICATION-LATERAL FLOW TO DETECT ACUTE CHAGAS DISEASE IN NEWBORNS

Clark Hamilton Cunningham

The University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

Chagas disease - caused by the parasite Trypanasoma cruzi - remains a significant public health issue throughout much of South and Central America. Congenital infection has become a greater proportion of new cases as vector-borne transmission has decreased, but most infants do not receive rapid diagnosis and treatment, exposing them to risk of long-term complications of Chagas disease. Treatment is highly effective in early life, but congenital Chagas disease is difficult to diagnose in settings without access to quantitative PCR, the most sensitive test in early infancy. One alternative to qPCR is recombinase polymerase amplification (RPA), an isothermal nucleic acid amplification method that can be coupled with a lateral flow (LF) readout to produce a simple, effective diagnostic assay. RPA-LF diagnostics have the additional advantages of producing results in under an hour and requiring minimal laboratory equipment or expertise. In this study, we adapted a previously described RPA-LF assay to detect acute T. cruzi infection in a cohort of 38 newborns who were positive by qPCR. Preliminary data shows a sensitivity of 89.5% (34/38), with two of the four false negatives in samples with very low parasite loads. These results demonstrate that RPA-LF can serve as an inexpensive, rapid, and sensitive clinical diagnostic for Chagas disease and other infectious diseases in low-resource settings.

389

IMMUNOTHERAPEUTIC EFFECT OF A NEW MULTI-EPITOPE CHIMERIC PROTEIN AS A VACCINE CANDIDATE FOR CHAGAS DISEASE

Cristina González-López[1], When-Hsiang Chen[2], Liliana Villanueva-Lizama[1], Miguel Rosado-Vallado[1], Christian Teh-Poot[1], Andrea Alfaro-Chacón[1], Jeroen Pollet[2], Kathryn Jones[2], Maria-Elena Bottazzi[2], Julio Vladimir Cruz-Chan[1]

[1]*Autonomous University of Yucatán, Merida, Mexico,* [2]*Baylor College of Medicine, Houston, TX, United States*

Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi* and it affects about 8 million people worldwide. Currently, there is no sterile cure, and approved drugs as nifurtimox and benznidazole are scarcely available and ineffective in chronic infection. Therefore, the development of a therapeutic vaccine has been proposed as a feasible alternative. Vaccinomics has emerged as a promising strategy that can save time and resources and it has proved to be useful against other infectious diseases. Thus, MHC class I epitopes predicted to elicit CD8+ T cells are essential to control *T. cruzi* infection. This study aimed to design, express and assay the immunotherapeutic effect of a new multi-epitope vaccine candidate based on ten nonameric epitopes linked to the well-characterized Tc24. C4 recombinant protein, as the backbone, resulting in the Tc24/10N multi-epitope protein. The recombinant plasmid encoding Tc24/10N was commercially synthesized, cloned and transformed into *E. coli* BL21 (DE3). Purification was performed using IMAC and the target protein was identified by Western blot. T. cruzi-infected mice were treated with Tc24/10N or Tc24.C4 alone in a 50-day acute phase model. We observed a 100% survival in the Tc24/10N-treated mice, significantly decreased parasitemia, and cardiac parasite burden compared to the Tc24.C4-treated mice. Moreover, Tc24/10N induced a higher stimulation index of CD8+ T cells producing IFN-γ, IL-4 and IL-17 cytokines. These results suggest that the addition of the MHC Class I epitopes to Tc24.C4 induces a stronger antigen-specific Th-1/Th-2/Th-17 immune response conferring protection to *T. cruzi* infection. This proof-of-concept study provides the insight that Tc24/10N is a promising candidate for the development of a Chagas disease immunotherapeutic vaccine.

390

GENETIC CHARACTERIZATION OF *TRYPANOSOMA CRUZI* FROM CHAGAS DISEASE PATIENTS

Stella M. Chenet[1], Rafael Tapia-Limonchi[1], Alan Oyarce[2], Jorge Fernández[2], Juan R. Tejedo[1], Maria I. Jercic[2]

[1]*Universidad Nacional Toribio Rodriguez de Mendoza, Chachapoyas, Peru,* [2]*Instituto de Salud Publica de Chile, Santiago, Chile*

Trypanosoma cruzi, the causative agent of Chagas disease, has a complex population structure with six main evolutionary lineages called discrete typing units (DTUs). So far, six DTUs have been described, from which TcII, TcV and TcVI are commonly found in humans. For this study, samples from thirteen patients with *T. cruzi* were characterized at the Institute of Public Health of Chile by sequencing the latosterol oxidase gene (TcSC5D). Eleven samples belonged to newborns or children below the age of two. The other samples belonged to a 61 year-old-man (patient A) diagnosed with HIV who developed brain trypanosomiasis and a 22 year-old-woman (patient B) with a chronic asymptomatic Chagas infection. All samples were positive by IgG ELISA, IFI and PCR. Sequencing analysis of the samples showed the presence of DTUs II, V and VI . Ten samples had monoclonal infections and three had polyclonal infections (more than one DTU per sample). From the monoclonal infections, two lineages belonged to TcII, six to TcV and two to TcVI. Patient A presented a TcVI infection while patient B presented a TcII infection. Two of the samples with polyclonal infections presented DTUs TcV/TcVI and the remaining sample DTUs V/VI. All monoclonal infections were present in Chilean patients; while the polyclonal infections were only found in newborns from Bolivian Chagasic mothers. We concluded that vertical transmission of more than one *T. cruzi* lineage is possible. Also, vector transmission of *T. cruzi*, increases the probability of polyclonal infections. On the other hand, monoclonal infections are expected in areas where the disease is under control such as in Chile. The genetic characterization of *T. cruzi* lineages could be applicable for molecular epidemiology to differentiate between autochthonous and introduced cases; moreover, we should further investigate the importance of the DTUs in the development of the host response early in the infection, which may influence the clinical progression of trypanosomiasis. Especially, if we consider that reactivation of Chagas disease occurs in immunosuppressed patients leading to severe disease.

391

EARLY ANTIPARASITIC TREATMENT PREVENTS PROGRESSION OF CHAGAS DISEASE: RESULTS OF A LONG-TERM CARDIOLOGICAL FOLLOW-UP IN A PEDIATRIC POPULATION

Nicolas Gonzalez[1], Samanta Moroni[1], Guillermo Moscatelli[1], Griselda Ballering[1], laura Jurado[1], Nicolas Falk[1], Andres Bochoeyer[1], Alejandro Goldsman[1], Hector Freilij[1], Eric Chatelain[2], **Jaime Altcheh**[1]

[1]*Hospital de Niños Ricardo Gutierrez, Buenos Aires, Argentina,* [2]*DNDi, Geneva, Switzerland*

Chagas disease (CD), caused by Trypanosoma cruzi, is distributed worldwide due to the migration of people from Latin America, where CD is endemic. Parasite persistence in tissues is crucial in the development and progression of Chagas long term complications such as cardiomyopathy, which occurs in 30% of untreated patients. Effective medications for acute CD have been available for decades, but there is little data about their effectiveness in preventing clinical progression of the disease. To this end, the parasitological, serological and cardiological long term evolution of a cohort of 95 children treated with either benznidazole or nifurtimox was analyzed. Median post-treatment follow-up time was 10 years. At the time of the last visit, a group of non-infected subjects was also studied as a control for cardiological studies. All treated patients tested showed T.cruzi qPCR negative results during follow up and T cruzi antibody titers decay in all treated patients while negative seroconversion was observed in 53/95 (56%).Cardiological evaluation revealed 24-hour Holter monitoring findings in 3/95 (3%) treated patients, but only one manifestation was considered compatible with CD (i.e. complete right bundle branch block). Minimal non pathological ECG findings were observed in 3/28 (10.7%) individuals of the non-infected control group. Myocardial contractility measured by 2D speckle tracking echocardiography was conducted in 79/95 (83%) and no alterations were observed in any of these subjects. Conclusions: Strong post-treatment parasiticidal effects and a very low incidence of cardiological lesions related to CD were observed in this follow-up study. Results suggest a protective effect of treatment with benznidazole or nifurtimox on the development of cardiological lesions and support the recommendation for early treatment of T cruzi infected children.

392

PEER-NAS-USAID 518 PROJECT: TOWARDS POC DNA DIAGNOSIS OF CUTANEOUS LEISHMANIASES IN MENA AND DEVELOPMENT IMPACT

Ikram Guizani[1], Yusr Saadi[1], Emna Harigua- Souiai[1], Imen Mkada[1], Imen Khammari[2], Ahmed S. Chakroun[1], Meryem Lemrani[3], Aïcha Kallel[4], Hejer Souguir[1], Rhea Coler[5], Hamed Chouaieb[2], Slaheddine Belhadj[4], Pierre AbiHanna[6], Seydou Doumbia[7], Nabil Haddad[8], Kalthoum Kallel[4], Akila Fathallah Mili[2], Steven Reed[5], Insaf BelHadj Ali[1]

[1]*Molecular Epidemiology & Experimental Pathology, Institut Pasteur de Tunis, Tunis, Tunisia,* [2]*Parasitology department, Hospital Farhat Hached, Sousse, Tunisia,* [3]*Institut Pasteur du Maroc, Casablanca, Morocco,* [4]*Parasitology department, Hospital La Rabta, Tunis, Tunisia,* [5]*Infectious Disease Research Institute, Seattle, WA, United States,* [6]*Rafik Hariri*

Hospital, Beirut, Lebanon, [7]Faculty of Medicine, University of Bamako, Bamako, Mali, [8]Faculty of Public Health, Lebanese University, Beirut, Lebanon

Cutaneous leishmaniases (CL) constitute major public health issues. Caused by multiple *Leishmania* species, they have diverse transmission cycles. More than 80 % of the cases occur in countries of the MENA region, where *L. major* or *L. tropica* are the predominant CL causing species, while *L. infantum* or *L. donovani* are involved to less extent. *Leishmania* species identification is central to control strategies, early alert on emergence, and timely and effective patient management. Notably, treatment algorithms are highly dependent on *Leishmania* species. Changes in eco-epidemiology of the disease, and even clinical presentations, are increasingly reported, due to climate variability, agriculture development, urbanization, population migration, and armed conflicts among other factors. As result, disease emergence in free areas and co-sympatry of the endemic species invalidate criteria such as clinical presentation or geographical distribution, for an accurate disease diagnosis and etiology. CL diagnosis, classically done by direct smear examination under a microscope (a tedious and poorly sensitive technique that requires experts in microscopy) does not identify the species. PCR assays used have drawbacks like long time to result, need for additional tests to identify the species, need for equipment and trained personnel. We aim for multiplex isothermal DNA amplification of the main CL species in MENA coupled to detection by lateral flow chromatography on a dipstick. Our approach spares precious samples, is rapid (<40min) and is appropriate for poor settings to support POC diagnosis. Our strategy includes R&D, proof of principle evaluation on CL samples from diverse origins, and development impact plans. It involves collaborators in the USA, Tunisia, Morocco, Lebanon and Mali.

393

DRIED BLOOD ON PAPER FILTER AS AN ALTERNATIVE FOR *TRYPANOSOMA CRUZI* ELISA SCREENING IN RURAL AREAS OF BRAZIL

Fabio De Rose Ghilardi, Lea Campos De Oliveira

São Paulo University, São Paulo, Brazil

Chagas Disease (CD) is a Tropical Neglected Disease (NTD) still prevalent in rural areas of Brazil, specially in the state of Minas Gerais (MG) where we estimate more than 1 million people living with this infection and only 2% being properly identified and treated. Most of them are from rural areas and were never tested due to lack of access to health care facilities. In order to allow a less complex screening for Chagas disease, we propose to validate an ELISA Chagas serology to be used in Dried Blood Spots (DBS) collected direct from the patient (by a digital punction) on a filter paper. A non inferiority trial as part of a validation study conducted in rural areas of Minas Gerais state, we collected matched finger-prick DBS and plasma samples from a convenience sample of 1890 adults (≥18 years) from São Francisco, Montes Claros between november 2018 and july 2019. We compare the results with gold standarts Chagas serology (anti-Tripanossoma cruzi) The study protocol were approved by the Universidade de Montes Claros e Universidade de São Paulo and all participants signed the written consent. In our study the statistical evaluation of an Elisa test performed in dried blood from paper filter revealed a sensitivity of 78%, specificity of 95% and a kappa coefficient of 0.86 appointing that reproducibility is acceptable, the paper test is a reasonable alternative for experimental or diagnostic purposes. A serological evaluation without venous puncture simplify collection, timing and handling of blood samples will improve screening of Neglected diseases. Accessibility to diagnostic and treatment are one of the main points to overcome the world impact of Neglected tropical diseases and it has been established as health care priority by WHO.

394

PATIENT PREFERENCES AND SATISFACTION WITH THERAPEUTICS FOR NEW WORLD CUTANEOUS LEISHMANIASIS

Rebecca Byler[1], Nancy Gore Saravia[2], Mayur Desai[1]

[1]*Yale University, New Haven, CT, United States,* [2]*CIDEIM, Cali, Colombia*

Treatment options for cutaneous leishmaniasis (CL) exhibit variable efficacy, safety, and convenience, which can limit patient adherence and adversely impact clinical outcome. This mixed methods study sought to comprehensively evaluate the patient experience during treatment in order to elucidate optimal CL treatment characteristics. In eighteen Colombian CL patients (15-60 years old, 50% female) who completed treatment, we assessed patient satisfaction with the treatment received, the impact of treatment on patient quality of life, and patient attitudes towards complementary and alternative therapeutic modalities if they were to be made available. During treatment, patients completed the Dermatology Life Quality Index (DLQI) and Treatment Satisfaction With Medicines Questionnaire (SATMED-Q). Upon treatment completion, we conducted semi-structuctured, in-depth interviews and administered an original questionnaire, the Cutaneous Leishmaniasis Therapeutic Preferences Survey (CLTPS). For 15 patients (83%), CL had a large or very large effect on the patient's quality of life, principally driven by disability and stigma from the presence of cutaneous lesions. Overall, patients experienced low treatment satisfaction (65.1/100, range 47.1-82.4), primarily due to perceived inconvenience of use and impact on daily activities. There was no difference in satisfaction between oral (miltefosine) and injection-based (Glucantime®) treatment options. However, treatment dissatisfaction was significantly associated with the presence of undesirable side effects. Patients expressed strong preferences for self-administration, shorter treatment duration, less invasive delivery routes, and improved scar management. Qualitative data indicate that patients combine biomedical (systemic) and traditional (topical) treatments to accelerate lesion closure and that misconceptions about the mortality rate of CL bolster perceived treatment satisfaction. This work establishes unmet patient needs and preferred therapeutic product characteristics that can be applied to design new, improved CL therapeutic modalities.

395

LABORATORY AND FIELD DIAGNOSTIC EFFICACY OF RECOMBINASE POLYMERASE AMPLIFICATION FOR CUTANEOUS LEISHMANIASIS

Alexandra Cossio[1], Thomas Shelite[2], Maxy De Los Santos[3], Jimena Jojoa[1], Maria del Mar Castro[1], Nancy Gore Saravia[1], Peter Melby[2], **Bruno L. Travi**[2]

[1]*Centro Internacional de Entrenamiento e Investigaciones Medicas, Cali, Colombia,* [2]*University of Texas Medical Branch, Galveston, TX, United States,* [3]*U.S. Naval Medical Research Unit 6, Lima, Peru*

Colombia and Peru have the highest number of cutaneous leishmaniasis (CL) cases in the Americas after Brazil. Highly sensitive and accurate diagnostic tests are restricted to centralized laboratories, while microscopy of stained lesion smears is the only available test in endemic regions. In general, microscopy has low sensitivity (≤70%). Therefore, efficacious point of care tests should be developed for resource-limited settings. Prior laboratory data of our diagnostic method based on recombinase polymerase amplification (RPA) and lateral flow reading (LF) indicated that the test had ≥90% sensitivity and specificity. We evaluated clinical samples from 226 patients from the Amazonian rain forest of Peru and 118 patients from the Pacific Southwest coast of Colombia (Tumaco) suspected of having CL. Samples from skin lesions were collected using 6 three-mm diameter FTA filter papers. Clinical samples from Peru were processed by RPA-LF in UTMB, Galveston and conventional PCR in NAMRU-6, Lima. In Colombia, samples were evaluated by RPA-LF in the local clinic (Tumaco) and in CIDEIM's lab in Cali and compared to a composite gold standard (smear+culture+biopsy+qPCR). In samples from Peru, the sensitivity of PCR was 94.5% and RPA-LF 92.2% having PCR+RPA-LF as reference test.

There was discrepancy between several samples that resulted positive by one test but negative by the other leading to low agreement. In Colombia, the sensitivity and specificity of RPA-LF processed in CIDEIM was 87% and 86% respectively compared with the composite gold standard. RPA-LF performed in Tumaco showed lower sensitivity (75%) but specificity comparable to the test performed in CIDEIM (86 vs. 89%). While there is still room for field optimization, RPA-LF fulfilled many of the POC features of a diagnostic test for CL as defined for the target product profile: a) applicability in health care facilities with no infrastructure or mobile lab, b) non-invasive sampling and c) visual reading of results. Overall, the results indicated that RPA-LF could be used as a complement to negative microscopy or as the only test where microscopy expertise is unavailable.

396

TRYPANOTHIONE REDUCTASE: POTENTIAL TARGET FOR DEVELOPING NEW THERAPEUTICS TO TREAT *LEISHMANIA DONAVANI*

Raman Mathur[1], Yash Gupta[1], Jesus Romero[1], Samuel Kwofie[2], Brijesh Rathi[3], Ravi Durvasula[1], **Prakasha Kempaiah**[1]

[1]*Loyola University Chicago Stritch school of Medicine, Maywood, IL, United States,* [2]*Loyola University Chicago Stritch school of Medicine and University of Ghana, Accra, Ghana, Maywood, IL, United States,* [3]*Loyola University Chicago Stritch school of Medicine and Hansraj College University Enclave, University of Delhi, Maywood, IL, United States*

Parasitic diseases remain to be a public health problem in many areas of the world, specifically tropical regions. *Leishmania* has become a disease that has been quite neglected in the areas of medicinal research. In our study, we focused on visceral leishmania (VL) also known as Kala-azar caused by *L. donovani*. There are an estimated 50,000-90,000 new cases every year with 20,000-30,000 deaths. Unlike other forms of leishmania, VL is life-threatening, causes enlargement of internal organs such as the spleen, liver, and bone marrow, and can lead to congestive heart failure. Presently, there are no approved vaccines available, and current drug treatments have adverse side effects. Here as part of target based drug discovery, we selected trypanothione reductase (TR), an essential *Leishmania* cellular enzyme and is crucial for its survival. To identify TR specific inhibitors, we screened n=1622 FDA approved drugs as part of SelleckChem bioactive libraries through CADD based virtual screening (Schrodinger-LLC 2020-1). The following compounds are identified to have targets in the FAD region of *L. donovani* TD: Flubendazole, Entospletinib, Lumacaftor, and Bezafibrate. These compounds are originally indicated for parasitic worm and cancer prophylactic. The binding energies for the compounds are -10.4, -9.7, -9.5, and -9.2 kcal/mol respectively. We further subjected the drug target complexes to MD simulations for 100ns in physiological conditions using Desmond software. The results revealed that these FDA drugs are good probes to explore the active site binding properties, which will aid in developing potent derivatives. The validation to test in-vitro on L. donovani cultures is underway. Overall, our discovery pipeline will enable us to understand the mechanism of action and further improve the lead drugs.

397

DIFFERENTIAL MODULATION OF NEUTROPHIL ACTIVATION BY ANTIMONY-RESISTANT AND SUSCEPTIBLE CLINICAL STRAINS OF *LEISHMANIA (V.) PANAMENSIS*

Olga Lucia Fernandez[1], Lady Giovanna Ramirez[1], Fabienne Tacchini-Cottier[2], Nancy Gore Saravia[1]

[1]*CIDEIM, Cali, Colombia,* [2]*Department of Biochemistry, WHO-Immunology Research and Training Center, University of Lausanne, Lausanne, Switzerland*

Emerging evidence has revealed that host innate responses may influence the response to anti-microbial drugs. Our recent studies have shown that *Leishmania* parasites selected *in vitro* for resistance to pentavalent antimony (SbV) induce significantly greater activation of both murine and human neutrophils, however, whether the differential effect of drug susceptibility phenotype extends to clinical strains is not known. This study evaluates the influence of SbV susceptibility phenotype of clinical strains of *L. (V.) panamensis* on the inflammatory response of human neutrophils. Neutrophils obtained from healthy donors (n = 3) were infected with SbV sensitive clinical strains (n = 10) or with intrinsically SbV clinical resistant strains (n = 10) isolated prior to treatment. Infected neutrophils were exposed to 32 ug SbV / mL, a concentration approximating the Cmax of SbV during treatment with meglumine antimoniate. The activation profile of neutrophils was evaluated based on expression of activation markers (CD66b, CD18, CD62L) using flow cytometry, production of reactive oxygen species (ROS) by luminometry, and quantification of NETs formation by PicoGreen fluorescence. These parameters were analyzed in relation to parasite susceptibility phenotype. Clinical strains having intrinsic resistance to SbV induced significantly lower ROS production compared to SbV sensitive clinical strains, both in the presence (P = 0.0314) and absence (P = 0.0073) of SbV. Expression of activation markers on the surface of neutrophils and NETs production did not show significant differences between the sensitive and resistant clinical strains under the conditions evaluated. However, exposure to SbV significantly reduced the NETs production (P = 0.0078), independently of parasite susceptibility phenotype. Activation of antimicrobial ROS production by human neutrophils is differentially modulated by clinical strains of *L. (V.) panamensis* having disparate susceptibility phenotypes for pentavalent antimony. Differential elicitation of innate response may contribute to therapeutic response as well as clinical outcome of infection.

398

IN VITRO LEISHMANIA INFANTUM AND TICK-BORNE BACTERIA CO-INFECTION MODEL IN A CANINE MACROPHAGE CELL LINE

Danielle Pessoa-Pereira, Breanna M. Scorza, Christine A. Petersen

University of Iowa, Iowa City, IA, United States

Visceral leishmaniasis (CanL) is a vector-borne zoonotic disease primarily caused by the protozoan *Leishmania infantum*, an obligate intracellular parasite classically transmitted via sandflies among reservoir host dogs and to nearby people. Our group has previously shown that exposure to tick-borne pathogens, such as *Ehrlichia spp.*, *Anaplasma spp.* or *Borrelia burgdorferi* can induce the progression of CanL in dogs. However how these tick-borne co-infections (TBC) affect the infected myeloid cell microbicidal response against *L. infantum* is not known. Considering that *L. infantum*, *Ehrlichia spp.*, *Anaplasma spp.* and *B. burgdorferi* are all intracellular pathogens, we hypothesized that host cells co-infected with *L. infantum* and tick-borne bacteria may display an increased, overly robust inflammatory phenotype. To investigate this, we established an *in vitro* co-infection model where a canine macrophage cell line, DH82 cells, was coinfected with *L. infantum* and tick-borne bacteria and used to evaluate synergistic effects on macrophage oxidative burst and cytokine production. *L. infantum* strain from naturally infected dogs was able to replicate in DH82 cells, which were also permissive for *Ehrlichia spp.* replication, and *B. burgdorferi* spirochete responses. We evaluated the effects of co-infection on (1) intracellular Leishmania replication; (2) inflammatory or anti-inflammatory cytokine production by ELISA; (3) reactive oxygen species production; and (4) expression of inflammatory-, antigen presentation-, and oxidative stress-related products. These underlying mechanisms of *L. infantum* and TBC promoted robust alterations in the myeloid cell inflammatory response promoting enhanced *L. infantum* infection.

399

RETROSPECTIVE STUDY OF SERODISCORDANT SUBJECTS FOR *TRYPANOSOMA CRUZI* INFECTION REVEALS DISTINCT SEROLOGICAL OUTCOMES AND INCREASED FREQUENCY OF NON-CLASSICAL MONOCYTES

Maria A. Natale[1], Maria J. Elias[1], Ana M. De Rissio[1], Maria G. Alvarez[2], Maria C. Albareda[1], Laura E. Fichera[1], Gonzalo Cesar[1], Constanza Lopez-Albizu[1], Karenina Scollo[1], Graciela Bertocchi[2], Bruno Lococo[2], Susana A. Laucella[1]

[1]*Instituto Nacional de Parasitologia Dr. Mario Fatala Chaben, Buenos Aires, Argentina,* [2]*Hospital Interzonal General de Agudos Eva Peron, San Martin, Argentina*

Serodiscordancy for *Trypanosoma cruzi* infection remains a challenge since individuals with inconclusive results more often do not receive clinical follow up and etiological treatment is not indicated. We have demonstrated that serodiscordant (SD) subjects have more functional, and of a higher magnitude, *T. cruzi*-specific T-cell responses, suggesting that some subjects exposed to *T. cruzi* might eventually resolve the infection. In this study, SD subjects (i.e., subjects with positive findings by only one serological test out of the three performed) were recruited, and their previous serological findings were examined in the database of the Instituto Nacional de Parasitologia Dr. Mario Fatala Chaben in Argentina. Three profiles emerged from the data collected, 1- subjects with sustained serodiscordant findings over time (median follow up= 4 y; range 1-16 y), 2- subjects who change from SD to seronegative status (median follow up= 3 y; range= 1-18 y), and 3- a group of subjects who showed positive serological findings and became SD or seronegative during follow up without the indication of etiological treatment (median follow up= 14 years, range 2-21 years). To further evaluate the immune status of SD subjects, we measured the frequency of different monocyte subsets in these subjects. SD subjects showed normal frequencies of classical and intermediate monocyte subsets as well as increased frequencies of non-classical monocytes which exert a potent anti-inflammatory function and wound healing. However, non-classical monocytes in SD express normal levels of the chemokine receptor CCR2. In conclusion, at least a proportion of the SD subjects had previously been seropositive for *T. cruzi* infection and display a resting status of reparative monocytes, further supporting that these subjects cleared the infection, and providing insights into the meaning of serodiscordancy for *T. cruzi* infection.

400

IMMUNIZATION WITH *LDCEN-/-* IS EFFICACIOUS IN MICE WITH CHRONIC *TRYPANOSOMA CRUZI* INFECTION

Sreenivas Gannavaram, Charles Thomas, Abraar Muneem, Nirmallya Acharyya, David Acosta, Alain Debrabant, Hira L. Nakhasi

US Food and Drug Administration, Silver Spring, MD, United States

Visceral leishmaniasis (VL) and Chagas disease are co-endemic in many parts of the world which may result in concurrent or prior exposure to both parasites in humans. Studies of co-infection of *Trypanosoma cruzi* and *Leishmania* have shown activation of distinct T cell differentiation profiles that result in clinical severity or control of pathogenesis. Towards developing a prophylactic vaccine against VL, we have tested centrin deleted live attenuated *Leishmania donovani* parasites (*LdCen-/-*) as potential vaccine. However, vaccination studies using *LdCen-/-* were performed mainly in naïve rodent models and immunological correlates of protection may not reflect the endemic conditions where simultaneous exposure to multiple pathogen infections is prevalent. In this study, we studied the effect of a prior exposure to *T. cruzi* Columbiana on the immune response and protective efficacy of the *LdCen-/-* vaccine. We infected C57Bl/6 mice intraperitoneally with 10^4 *T. cruzi* parasites and monitored the parasitemia to establish chronic infection. Immunological analysis showed that characteristics associated with a chronic *T. cruzi* infection such as CD80+CD64+ pro- and CD206lo CD200Rlo anti-inflammatory splenic macrophages were recapitulated. Immunization of C57Bl/6 mice chronically infected with *T cruzi* followed by challenge with virulent *L. donovani* parasites showed that *LdCen-/-* parasites induced potent protective immunity comparable to naïve immunized hosts. Analysis of the immune response from spleen and lymph nodes revealed that high abundance of IL-17 and IL-10 secreting T cells in chronic Chagas mice did not impact the protective immunity as revealed by comparable parasite clearance upon challenge infection with virulent *L. donovani*. The frequency of multifunctional IFN-g, IL-2 and TNF secreting CD4 and CD8 T cell populations was comparable between naïve immunized mice and chronically infected Chagasic immunized mice following virulent challenge with *L. donovani*. These results suggest that immunization with *LdCen-/-* parasites is equally efficacious in individuals with chronic Chagas infection as in naïve vaccinees.

401

DIET IMPACTS LIVER ARCHITECTURE, PARASITE BURDEN, AND TRANSCRIPT EXPRESSION DURING *LEISHMANIA INFANTUM* INFECTION

Mark Wacker[1], Upasna Dixit[1], **Ellen Kiser**[2], Hemali Batra-Sharma[3], Yani Chen[4], Mary Wilson[5]

[1]*Department of Internal Medicine at the University of Iowa, Iowa City, IA, United States,* [2]*Department of Microbiology and Immunology at the University of Iowa, Iowa City, IA, United States,* [3]*Carver College of Medicine at the University of Iowa, Iowa City, IA, United States,* [4]*Department of Internal Medicine at the University of Iowa and Veterans' Affairs Medical Center, Iowa City, IA, United States,* [5]*Department of Internal Medicine, Department of Microbiology and Immunology, and Carver College of Medicine at the University of Iowa and Veterans' Affairs Medical Center, Iowa City, IA, United States*

South American visceral leishmaniasis (VL) is a fatal disease caused by *Leishmania infantum* (*Li*) in Brazil. And in Brazil the incidence of obesity and cardiovascular disease is currently rising as diet and behavior become more Westernized. Coincident with these changes, the ratio of asymptomatic to symptomatic infections with *Li* is rising. We hypothesized that the outcome of *Li* infection is influenced by changes in dietary fat and cholesterol. To test this, we fed BALB/c mice a control, high fat, or high fat-high cholesterol diet. Four weeks later, half the mice were infected with *Li* expressing firefly luciferase. Using *in vivo* imaging, we quantified parasite burden by quantifying the amount of light emitted from specific organs. The data revealed that parasite loads in the liver were significantly increased in mice fed the high fat diet and significantly decreased in mice fed the high fat-high cholesterol diet compared to the control diet. After 8 weeks of infection (12 weeks of diet), mice were euthanized, and RNA was extracted from liver tissues. An Illumina microarray revealed significant changes in the immune microenvironment and increased expression of metabolic transcripts in the experimental diets. Consistently, histologic sections revealed a large infiltrate of neutrophils in mice on the high fat-high cholesterol diet. These data have led us to hypothesize roles for fatty acid and steroid biosynthetic pathways in the inflammatory response to and outcome of *Li* infection. Metabolic changes in the liver and other organs may play a role in the progression of this parasitic infection.

402

DIABETES MELLITUS MODIFIES THE CLINICAL PRESENTATION OF CUTANEOUS LEISHMANIASIS AND IMPAIR RESPONSE TO THERAPY IN PATIENTS WITH ATYPICAL LESIONS

Alexsandro Souza Lago[1], Augusto M. Carvalho[1], Luiz Henrique Guimarães[2], Jamile Lago[1], Lucas Carvalho[3], **Edgar M. Carvalho**[3]

[1]*Federal University of Bahia, Salvador, Brazil,* [2]*Federal University of the South of Bahia, Itabuna, Brazil,* [3]*Oswaldo Cruz Foundation, Salvador, Brazil*

It is estimated that 480 million people have diabetes mellitus (DM) in the world, a disease that is associated with impairment in neutrophil and monocyte function. Cutaneous leishmaniasis (CL) caused by *Leishmania braziliensis* is characterized by a well-limited ulcer with raised borders. A typical CL lesions as vegetative, large and multiple nodular lesions only

occurs in about 1% of the patients. CL affects predominantly young adults and there is a lack of studies of the association between DM and CL. In the present study we evaluate the influence of DM on clinical manifestations, immune response and in the treatment of CL patients. Participants were 32 DM patients with CL with age between 18 to 60 years and 32 patients with CL without DM matched by age followed at the Corte de Pedra Health Post in Bahia, Brazil. The diagnosis of CL was performed by documentation of DNA of *L. braziliensis* by PCR in the lesion biopsy. All patients were treated with pentavalent antimony, glucantime (Sanofi-Aventis) at the dose of 20mg/Kg of weight for 20 days. There was no difference between the groups regarding gender, illness duration, size and location of the lesions, frequency of satellite lymphadenopathy and lymph node size. Patients with CL and DM were older than patients without DM and the cure rate 66% and 56% respectively was similar in both groups (P>.05). We found no influence of blood sugar levels and the type of diabetes in the response to therapy. While all patients with only CL had typical ulcers, 38% of the patients with DM and CL had large not well-limited superficial ulcers. High levels of TNF and IL-1β were detected in supernatants of mononuclear cells stimulated with leishmania antigen in patients with DM and atypical cutaneous lesions. Failure to therapy was observed in 72% of the patients with DM and atypical CL lesions and in 43% of the patients with typical lesions. DM modify the clinical presentation, enhances pro-inflammatory cytokine production and impair response to antimony therapy.

403

INCREASED PATHOLOGY IN *LEISHMANIA MAJOR* INFECTION MEDIATED BY DYSBIOTIC SKIN MICROBIOTA IS DEPENDENT ON IL-1B AND IL-17 PRODUCTION

Augusto M. Carvalho[1], Phillip Scott[2]

[1]*Federal University of Bahia, Salvador, Brazil,* [2]*Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, United States*

Microbiota play an important role in skin physiology, promoting inflammatory processes, skin immunity against microbes and tissue repair. We previously reported that a pre-existing dysbiotic skin microbiota exacerbates pathology in *L. major* infection. Here, we evaluated the mechanisms involved in this increased pathology caused by dysbiotic skin microbiota. To assess this, a dysbiotic skin mouse model was created by topical associations with *Staphylococcus epidermidis*. Dysbiosis was confirmed by counting the colony-forming unit (CFUs). Dysbiotic mice infected with *L. major* exhibited larger lesions than controls without any difference in parasite load or IFN-γ production by T cells. The enhanced lesion size in *L. major* infected dysbiotic mice was associated with an increase in neutrophil recruitment, IL-1β and IL-17 production. Both CD4+ and double negative T cells were the major IL-17 source. To assess the role of IL-1β we treated dysbiotic mice with anti-IL-1β monoclonal antibody and observed a reduction in neutrophil recruitment and IL-17 production. Importantly, this treatment had no effect on the parasite burden or IFN-γ responses, but prevented the dysbiosis-driven pathology in *L. major*-infected mice. Our results indicate that a skin dysbiosis enhances disease in an IL-1β dependent fashion, suggesting that blocking this cytokine may aid in ameliorating some of the pathology associated with cutaneous leishmaniasis.

405

CURRENT DIAGNOSIS AND BIOMARKER DISCOVERY FOR THE THREE MAJOR *SCHISTOSOMA* SPP.: *S. MANSONI, S. HAEMATOBIUM*, AND *S .JAPONICUM*

John C. Noh, Yong Wang, Olga Stuchlik, Matthew S. Reed, Jan Pohl, Sukwan Handali

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

The CDC Parasitic Diseases CLIA laboratory offers human antibody detection tests for *Schistosoma mansoni*, *S. haematobium*, and *S. japonicum*. The assays used rely on highly purified native antigens produced in-house. However, production of these antigens is laborious, requiring sophisticated methods, highly qualified labor, expensive and subject to high inter-batch variation. To control the potential for batch-to-batch variability, these processes incorporate highly stringent quality and validation controls. Therefore, we are seeking to develop assays that are more robust and have potential to be made available to other laboratories by replacing the native antigens with recombinant peptides. We are using size-exclusion chromatography, 1D gel electrophoresis, and tandem mass spectrometry as a workflow to discover biomarkers for serodiagnosis of these three *Schistosoma* spp. Based on previous results, we are focusing on the following target proteins for each Schistosoma species: gp25 and gp29 for *S. mansoni,* gp23 for *S. haematobium,* and gp 18-21 for *S. japonicum*. Whole extracts of each adult worm were initially sonicated and pelleted, then examined and tested for sero-reactive proteins. Our initial analyses of whole parasite extracts showed abundance of the 7 proteins of interest in the supernatants as well as additional potential proteins. We will next separate these supernatants by size-exclusion chromatography, then electrophorese and blot onto nitrocellulose membranes, and test using positive, cross reactive, and negative reference sera. The reactive protein bands will be subjected to decarboxylation to break apart glycoproteins. Fractions containing the non-affected bands will be analyzed by mass spectrometry for protein sequencing, eventually leading to identification of new candidate biomarkers which could be produced as recombinant proteins or synthetic peptides. If successful, the replacement of native materials with new recombinant peptides would be a significant improvement over current methods and may allow wider access to testing while also lowering the operational cost to produce the antigens.

407

DEVELOPMENT OF GENUS- AND SPECIES-SPECIFIC ANTIGENS FOR DETECTION OF ANTIBODIES AGAINST *SCHISTOSOMA* SPP. INFECTIONS

Sylvia Ossai[1], Yong Wang[1], Sukwan Handali[2], Austin Newsam[1], Rafael R. de Assis[3], Jeffrey M. Bethony[4], Rodrigo Correa-Oliveira[5], Philip T. LoVerde[6], Philip L. Felgner[3], W. Evan Secor[2]

[1]*Synergy America, Inc., Atlanta, GA, United States,* [2]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [3]*University of California Irvine, Irvine, CA, United States,* [4]*George Washington University, Washington, DC, United States,* [5]*Instituto Rene Rachou, FIOCRUZ, Belo Horizonte, Brazil,* [6]*School of Medicine, University of Texas Health Science, San Antonia, TX, United States*

Diagnosis of schistosomiasis caused by *Schistosoma haematobium, S. japonicum,* and *S. mansoni* using microscopic identification of eggs in stool or urine lack sensitivity because eggs may be passed intermittently or in small amounts. Antibody detection using ELISA or multiplex bead assays (MBA) can be useful alternatives to detect schistosome infection at the individual or population level. We aimed to identify recombinant proteins that could be used for schistosome genus- or species-specific antibody detection. To accomplish these goals, we applied two strategies: identification of new target antigens using schistosome antigen arrays and using species homologs from previously described antigens. The peptide array identified 6 putative target proteins (annexin, saposin, and hypothetical proteins 1 through 4) that were subsequently expressed and purified. Two of these recombinant proteins, saposin and hypothetical protein 3 (hypo3), were produced in adequate quantity and were recognized by sera from *Schistosoma* spp.-infected individuals. Saposin was detected by persons infected with any of the 3 main *Schistosoma* spp., while hypo3 was only recognized by persons with *S. mansoni* or *S. japonicum* infections. We also expressed and purified 7 antigens (Sm25, Sm29, Sm22.3, Sh29, LGG Sh, LGG Sj and Sj29) whose protein sequences were derived from homolog proteins of previous studies. Sm25, Sm22.3, Sh29 and LGG Sh were recognized by sera from persons with *S. mansoni* and *S. japonicum,* while LGG Sj was strongly recognized only by persons with *S. japonicum* infections. Sm25 and LGG Sj coupled to Magpix beads provided strong signals for detecting antibodies in sera from infected individuals in an MBA format. Sm25 is specific for detecting *S. mansoni* infections and LGG Sj is specific for detecting *S. japonicum* infections;

we have not yet identified recombinant antigens specific for detecting *S. haematobium* infections. More work is being done into expressing and purifying the other proteins, and ultimately develop MBA strategies for better detection of genus- and species-specific antibodies against *Schistosomiasis* spp. infections.

409

DIAGNOSIS OF *SCHISTOSOMA MANSONI* INFECTIONS IN ASYMPTOMATIC ERITREAN MIGRANTS BY STOOL PCR AND THE DETECTION OF CIRCULATING ANODIC ANTIGEN (CAA) IN URINE AND SERUM

Pytsje Hoekstra[1], Afona Chernet[2], Claudia J. de Dood[3], Eric A. Brienen[4], Paul L. Corstjens[3], Beatrice Nickel[2], Linda J. Wammes[5], Govert J. van Dam[4], Andreas Neumayr[6], Lisette van Lieshout[4]

[1]*Leiden University Medical Center, Leiden, Netherlands,* [2]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [3]*Department of Cell and Chemical Biology, Leiden University Medical Center, Leiden, Netherlands,* [4]*Department of Parasitology, Leiden University Medical Center, Leiden, Netherlands,* [5]*Department of Medical Microbiology, Leiden University Medical Center, Leiden, Netherlands,* [6]*Swiss Tropical and Public Health Institute, b, Switzerland*

The high number of African migrants coming from *Schistosoma* endemic regions and arriving in Europe justifies the question whether they should be screened for *Schistosoma* infection and which diagnostic test should be used in this specific population. Here, we explore the diagnostic value of genus specific *Schistosoma* DNA and Circulating Anodic Antigen (CAA) detection in a group of asymptomatic migrants from Eritrea who arrived recently in Switzerland. Test results were compared with already available stool microscopy and urine Point-of-Care Circulating Cathodic Antigen (POC-CCA) data, which showed 24% and 29% *S. mansoni* positives, respectively. Single urine, stool and serum samples were available from 92 individuals, and from 23 individuals one year after praziquantel (PZQ) treatment. Urine and stool samples were tested by real-time PCR for the detection of *Schistosoma* DNA, while the ultra-sensitive and highly specific UpConverting Phosphor labelled Lateral Flow (UCP-LF) CAA test was used on urine and serum samples to determine *Schistosoma* CAA levels. At baseline, urine PCR was negative in all individuals while 25% were positive by stool PCR. CAA was detected in 40/92 (44%) of urine and in 37/92 (40%) of serum samples. The serum CAA test was able to confirm all microscopy positives except two cases which were only positive by microscopy and negative by all other diagnostic tests. Additionally, those who were positive at baseline and tested after treatment showed a significant reduction in infection prevalence and intensity, in particular when tested by serum CAA. Our findings show that conventional microscopy and POC-CCA lack sensitivity to detect all active *Schistosoma* infections in this study population. The accuracy of stool PCR was similar to conventional microscopy, indicating that also this method is not sensitive enough in this setting. The serum CAA test seems to be the most sensitive method for screening of active *Schistosoma* infections in newly arriving asymptomatic migrants. Although tested in only a small number of individuals, the data also confirm CAA to be a suitable genus specific marker for monitoring PZQ treatment efficacy.

410

DEVELOPMENT OF NOVEL THERAPEUTICS AGAINST SCHISTOSOMIASIS

Sevan N. Alwan[1], Rolando Trevino, Jr.[1], Claudia Moreno Romero[1], Alexander B. Taylor[1], Jayce Rhodes[2], Michael Tidwell[2], Stanton McHardy[2], Philip T. LoVerde[1]

[1]*University of Texas Health Science Center, San Antonio, TX, United States,* [2]*University of Texas at San Antonio, San Antonio, TX, United States*

Human schistosomiasis is a neglected tropical disease caused by parasitic worms. It affects over 250 million people globally. Most human infections are caused by *Schistosoma mansoni, S. haematobium,* and *S. japonicum.* Currently there is only one method of treatment for human schistosomiasis, the drug praziquantel. Constant selection pressure has caused a serious concern for a rise in resistance to praziquantel leading to the necessity for additional pharmaceuticals, with a distinctly different mechanism of action, to be used in combination therapy with praziquantel. Previous treatment of *Schistosoma mansoni* included the use of oxamniquine (OXA), a prodrug that is enzymatically activated by a Sulfotransferase, an enzyme produced by *S. mansoni* (*Sm*SULT). Although sulfotransferases are produced by *S. haematobium* and *S. japonicum,* OXA is not effective against these two species. By using information from the crystal structure of *Sm*SULT bound to OXA, 250 OXA derivatives were designed and tested *in vitro* against the adult parasites. We were able to identify effective derivatives that kill *Schistosoma mansoni* (85%), *S. haematobium* (40%) and *S. japonicum* (83%). Recently, we identified 40 mg/kg of CIDD-149830 as an effective derivative that can kill all three schistosome species (100%) within 7 days *in vitro.* A dose-dependent study demonstrated that 20 mg/kg killed 100% *S. mansoni* but not *S. haematobium* or *S. japonicum*.

411

IMPACT OF PERIODIC SELECTIVE PZQ TREATMENT ON *SCHISTOSOMA MANSONI* INFECTION AS MONITORED BY KATO-KATZ AND POC-CCA IN A SCHISTOSOMIASIS ENDEMIC COMMUNITY IN THE DEMOCRATIC REPUBLIC OF CONGO

Clémentine Roucher[1], Joule Madinga[2], Marie Roger Hermy[1], Sylvie Linsuke[2], Linda Paredis[1], Pytsje Hoekstra[3], Govert van Dam[3], Lisette van Lieshout[3], Placide Mbala[2], Pascal Lutumba[2], Katja Polman[1]

[1]*ITM, Antwerpen, Belgium,* [2]*Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of the Congo,* [3]*Leiden University Medical Center, Leiden, Netherlands*

Reliable and accurate diagnostic methods are key for monitoring drug efficacy and re-infection. The point-of-care circulating cathodic antigen test (POC-CCA) has been proposed as a diagnostic alternative for the Kato-Katz (KK) to detect *Schistosoma mansoni* (*Sm*) infections. In this study we compared POC-CCA with KK for assessing the impact of periodic selective treatment on *Sm* infection in a rural schistosomiasis endemic community in DRC. A cohort of 250 individuals (1-80 years) was followed up at 3-week intervals for 3 months. At baseline and for each round, two stool samples and at least one urine sample were collected and subjected to KK and urine filtration (UF) for the microscopic detection of Sm and S. haematobium (Sh) eggs, respectively. In addition, an aliquot of urine was tested for Sm infection by POC-CCA and an aliquot of stool was tested for Schistosoma spp by PCR. At each round, those who were positive by microscopy were treated with 40 mg/kg PZQ. At baseline, 71.6% of the participants were *Sm* positive by KK and 71.6% by POC-CCA; agreement between the results of both tests was moderate (Kappa = 0.508). In this group, 68.8% were *Sh* positive by UF and 80.4% were *Schistosoma* positive by PCR. After the first round of treatment, 94.7% of all individuals who were KK positive at baseline were cured; after the second round this was 100%. Starting from those who were POC-CCA positive at baseline and treated, only 69.9% were cured. After the third round of treatment, the cure rate (CR) increased to 98.1%. Based on UF, CR increased from 65.4% after the first round to 94.3% after three rounds of treatment. For those who were PCR-positive at baseline, 82.7% were cured after one treatment round. No PCR data were available for round 2 and 3. Our results indicate that periodic selective treatment with PZQ is effective in reducing the number of schistosome infections in an endemic community in DRC. Based on KK, two rounds of treatment were sufficient to achieve a CR of 100%, while POC-CCA and UF still detected infections after three rounds. These findings have important implications as to what diagnostic and treatment strategies to implement when proceeding towards elimination.

412

WATER CONTACT ACTIVITIES, SNAIL INTERMEDIATE HOSTS, CERCARIAL SHEDDING AND THE SWIMMING BEHAVIOR OF THE FURCOCERCUS CERCARIA OBTAINED FROM YADAKUNYA PART OF JAKARA DAM, KANO STATE, NIGERIA

Rabi Suraj Duwa

Nigeria police academy, Wudil, Nigeria

Water contact activities and the presence of snail intermediate hosts are important for the transmission of schistosomiasis. Schistosomiasis is a neglected tropical disease caused by infections with trematodes of the genus Schistosoma. Snails of the genus *Bulinus* and *Biomphlaria* are the intermediate hosts of this trematode where the asexual stage of the life cycle takes place while the sexual stage takes place in man, the definitive host. This study reports water contact activities, the presence of different snail intermediate hosts and different furcocercus cercaria in infected snails from Ungoggo part of Jakara dam, Kano state North-Western Nigeria. This part of the dam serves for fishing and crops farming during the rainy and dry season (irrigation) so there is water contact activities taking place every day. People also cross to neighbouring villages by boat. Snails were collected by hand picking only from water hyacinth and directly from the water. Different *Bulinus* and *Lymnea* species were encountered in addition to Melanoides and Bithynia species. Cercarial shedding method was used to recover cercariae from snails and microphotographs and films of cercaria were taken using a ToupView digital camera attached to a microscope. Different species of Bulinus were encountered and many of them were shedding cercariae. Five different types of furcocercus cercaria were recovered. The swimming behaviour of the two furcocercus cercariae were also recorded (video) and discussed. The backwardly curvation of the furca of these cercariae and the separation of the body from the tail was observed under pressure of the coverslip on the slide. One thing worthy of note is that there were no *Biomphlaria* species in all the parts of Jakara dam and that *Bulinus* species were emitting many cercariae suspected to be that of *Schistosoma mansoni* during this study. This is the first report of different types of Furcocercus cercariae in this part of the dam. Jakara dam is one of the most polluted dams in Africa.

413

HIGH-RISK WATER CONTACT BEHAVIOUR AND INCREASED INFECTION RISK AMONG SCHOOL-AGED CHILDREN WITH RAPID *SCHISTOSOMA MANSONI* (RE)INFECTION, LAKE VICTORIA, UGANDA

Suzan Catherine Marie Trienekens[1], Christina L. Faust[1], Fred Besigye[2], Lucy Pickering[1], Poppy H. Lamberton[1]

[1]*University of Glasgow, Glasgow, United Kingdom,* [2]*Vector Control Division, Ministry of Health, Kampala, Uganda*

Annual mass drug administration with praziquantel has been successful in reducing schistosomiasis in some high-endemic areas but persistent transmission hotspots (PHS) have been identified across sub-Saharan Africa, including Uganda. This mixed-methods study aimed to better understand variation in water contact behaviours and infection risk in school-aged children (SAC) within a PHS to inform additional control efforts. Data were collected in Bugoto, Mayuge District, Uganda. Eight children with no/very low infection (CLI) and eight children with high baseline and reinfection (CHI) were recruited from a longitudinal SAC cohort. Structured day-long observations were undertaken with each child individually in two seasons in 2018. Observations included location and type of water contact, duration, frequency and level of submersion. In all identified water contact sites, four snail surveys were conducted quarterly over one year. All observed *Biomphalaria* snails were collected, counted and monitored for *S. mansoni* cercarial shedding for three weeks. Water contact was frequent and occurred in home, school and community settings for domestic, personal care, recreational, religious and commercial purposes. Only CHI were observed to swim (up to 4x per day) and fetch water commercially (up to 5x per day). CHI also contacted water at more sites than CLI. Households of CLI collected rainwater more often. A total of 9,457 *Biomphalaria* snails were collected from 10 sites. Three adjacent lake sites with the highest abundance, mainly *B. choanomphala* (64%), were contacted more by CHI. Over the year, only six snails were found shedding cercariae, of which four from sites only contacted by CHI. The findings of this mixed-methods study suggest that CHI perform more high-risk water contact behaviour and access water sites with higher *Biomphalaria spp*. abundance, demonstrating that specific water contact behaviours and environments help explain variation in risk within a PHS. Targeted behaviour change, vector control, and safe water supply to complement mass drug administration should reduce reinfection in SAC living in these PHS.

414

COMPARATIVE VECTORIAL COMPETENCE OF *BIOMPHALARIA SUDANICA* AND *B. CHOANOMPHALA,* SNAIL HOSTS OF *SCHISTOSOMA MANSONI* IN THE TRANSMISSION HOTSPOTS OF LAKE VICTORIA BASIN IN WESTERN KENYA

Martin W. Mutuku[1], Martina R. Laidemitt[2], Johannie Spaan[3], Ibrahim N. Mwangi[1], Horace Ochanda[4], Michelle Stainauer[3], Eric S. Loker[2], Gerald Mkoji[1]

[1]*Kenya Medical Research Institute, Nairobi, Kenya,* [2]*University of New Mexico, Albuquerque, NM, United States,* [3]*Western University of Health Sciences, Lebanon, OR, United States,* [4]*University of Nairobi, Nairobi, Kenya*

Schistosoma mansoni which causes intestinal schistosomiasis continues to be a public health concern in the Lake Victoria basin in Kenya, with *Biomphalaria sudanica* (a shoreline inhabiting snail) and *B. choanomphala* (a deep-water snail) playing roles in transmission. A recent study showed that *B. sudanica* was abundantly present in all study villages in the lake, but *B. choanomphala* was significantly more abundant in villages known to be persistent transmission hotspots. The present study investigated compatibility of *B. sudanica* and *B. choanomphala* with *S. mansoni.* A reciprocal cross infection experiment used 2-months old F1 generation *B. sudanica and B. choanomphala* which were exposed to either 1, 5 or 10 sympatric or allopatric *S. mansoni* miracidia. 3 weeks post-exposure (PE) and weekly thereafter, the snails were counted and screened for cercariae, and at 7 weeks PE, the total cercariae shed during a 2 hr period was determined. Pre-patent periods for *S. mansoni* in both *B. sudanica* and *B. choanomphala* were similar, most snails started shedding cercariae 5 weeks PE. Infection rates were significantly higher in *B. choanomphala* (12.2-80.9%) than in *B. sudanica* (5.2-18.6%) at each dose, regardless of miracidia source (P <0.0001). Overall, the odds of a snail becoming infected with 5 or 10 miracidia were significantly higher (494% and 569% respectively) than the odds of being infected with 1 miracidium, (P <0.0001). On average, *B. choanomphala* produced more cercariae (456) than *B. sudanica* (237.5). These results suggest *B. choanomphala* is a more efficient transmitter of *S. mansoni* than *B. sudanica*. *B. choanomphala*'s role in transmission seems under-appreciated, because dredging is an inefficient means of sampling its habitats, and therefore, easily underestimates its population size. Supported by NIH Grant # R37AI101438.

415

EFFECTS OF AGROCHEMICAL POLLUTION ON SCHISTOSOMIASIS ECOLOGY AND EPIDEMIOLOGY

Christopher M. Hoover[1], Samantha L. Rumschlag[2], Luke Strgar[1], Arathi Arakala[3], Manoj Gambhir[4], Giulio A. de Leo[5], Susanne H. Sokolow[5], Jason R. Rohr[2], Justin V. Remais[1]

[1]*UC Berkeley, Berkeley, CA, United States,* [2]*University of Notre Dame, Notre Dame, IN, United States,* [3]*RMIT University, Melbourne, Australia,* [4]*NA, Melbourne, Australia,* [5]*Stanford University, Stanford, CA, United States*

Agrochemical pollution of surface waters is a growing global environmental challenge. As intensive, industrial agricultural practices expand in developing areas, the effects of agrochemical pollution on the transmission of environmentally mediated infectious disease will

become more common. We report results from recent mesocosm, field, and systematic review studies that together provide substantial evidence that agrochemical pollution affects the transmission dynamics of schistosomiasis through complex ecological pathways. Bottom-up stimulation of algal resources by fertilizers and herbicides such as atrazine can increase the basic reproduction number, R_0, by as much as 300% by increasing resource availability for intermediate host snails. In areas where aquatic arthropod predators of intermediate host snails suppress transmission, common pyrethroid and organophosphate insecticides may also increase transmission through their toxic effects on predators of snails and free-living aquatic larval stages of schistosomes. To identify areas of overlap between agricultural intensification and schistosomiasis endemicity, we combine remote sensing approaches to identify agricultural areas with the schistosomiasis data from the Global Neglected Tropical Diseases Database (gntd.org) Environmentally relevant concentrations of agrochemicals alter schistosomiasis transmission through direct and indirect effects on intermediate host and parasite densities. As industrial agricultural practices expand in areas where schistosomiasis is endemic, strategies to limit increases in transmission due to agrochemical pollution should be developed and pursued.

416

PREVALENCE, EXPOSURES, AND RISK FOR SCHISTOSOMIASIS IN THE U.S. MILITARY

Alyssa R. Lindrose[1], Indrani Mitra[2], Jamie Fraser[2], Patrick W. Hickey[3], Edward Mitre[4]

[1]*Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences; The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., Bethesda, MD, United States,* [2]*Infectious Disease Clinical Research Program, Department of Preventive Medicine and Biostatistics, Uniformed Services University of the Health Sciences, Bethesda, MD, United States,* [3]*Department of Pediatrics, Uniformed Services University of the Health Sciences, Bethesda, MD, United States,* [4]*Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, MD, United States*

Schistosomiasis is a parasitic worm infection estimated to infect over 200 million people worldwide. A previous study conducted by our group found there were 317 schistosomiasis diagnoses in the U.S. Military Health System between Fiscal Year 2012-2018. The current study consisted of an in-depth case review to investigate travel history, exposures, risk factors, and treatment of schistosomiasis infections. We evaluated 42 cases that were validated by laboratory testing (positive *Schistosoma sp.* IgG, n=19, and/or eggs identified on biopsy or stool/urine ova & parasite test, n=32). Seven cases were identified as *S. haematobium*, 12 as *S. japonicum*, 5 as *S. mansoni*, and in 18 cases the species was not or could not be identified. The most common endemic area of travel was the Philippines (n=13). Of these 13 patients, 12 were native to the country. Additionally, 13 other patients were assumed to have acquired schistosomiasis in their native country. Other areas of travel identified as the most likely site of acquisition included Vietnam, South East Asia (not further specified), Ghana, Togo, Sudan, and various other regions of Africa. Eight patients did not have travel history reported in the medical record. Only 14 patients (33%) reported a history of freshwater exposure. Of note, 29 patients (69%) were diagnosed by biopsy specimen, indicating the infection had persisted long enough to cause significant pathology. While schistosomiasis is often asymptomatic, chronic infection and the resulting inflammation can result in serious complications, including bowel polyps, liver fibrosis, and urinary tract damage. Nearly all (95.2%) cases had appropriate treatment prescribed (praziquantel) as documented in the medical record. In cases where the species was not definitively diagnosed, providers prescribed the highest dose treatment or the appropriate treatment for the species endemic to the area of travel. This study suggests that military personnel who travel to or are native to schistosomiasis-endemic countries may be at risk for infection, and schistosomiasis serology screening may be indicated upon return to the U.S. to ensure timely treatment.

417

POTENTIAL IMPACT OF CLIMATE CHANGE ON SCHISTOSOMIASIS: A GLOBAL ASSESSMENT ATTEMPT AND ADAPTATION CASE STUDY IN CHINA

Guo-Jing Yang[1], Robert Bergquist[2]

[1]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [2]*Geospatial Health, Ingerod, Sweden*

Based on an ensemble of five global circulation models (GCMs), four representative concentration pathways (RCPs) and several ongoing and planned Coupled Model Intercomparison Projects (CMIPs), the Intergovernmental Panel on Climate Change (IPCC) predicts that the global, average temperatures will increase by at least 1.5ºC in the near future and more by the end of the century if GHG emissions are not genuinely tempered. While the RCPs are indicative of various amounts of greenhouse gases (CHGs) in the atmosphere the CMIPs are designed to improve the workings of the GCMs. We chose RCP4.5 which represents medium GHG emission increase and CMIP5, the most recently completed CMIP phase, combining this meteorological model with a biological counterpart model accounting for replication and survival of the snail intermediate host as well as maturation of the parasite stage inside the snail at different ambient temperatures. The potential geographical distribution for the three main schistosome species: *Schistosoma japonicum*, *S. haematobium* and *S. mansoni* was investigated with reference to their different transmission capabilities at the monthly mean temperature, the maximum temperature of warmest month(s) and the minimum temperature of the coldest month(s). The set of six maps representing the predicted situations in 2021-2050 and 2071-2100 for each species, mainly show increased transmission areas for all three species but they also leave room for potential shrinkages in certain areas.

418

KNOWLEDGE LEVEL OF URINARY SCHISTOSOMIASIS, A POTENTIAL BOTTLENECK TO DISEASE CONTROL IN THE HYPERENDEMIC HEALTH DISTRICT OF KENIEBA HEALTH DISTRICT, KAYES, MALI

Abdoul Fatao Diabate[1], Moussa Sangare[1], Yaya Ibrahim Coulibaly[2], Housseini Dolo[3], Siaka Yamoussa Coulibaly[1], Lamine Diarra[1], Salif Seriba Doumbia[1], Ilo Dicko[1], Abdallah Amadou Diallo[1], Lamine Soumaoro[1], Michel Emmanuel Coulibaly[1], Seydou Doumbia[4]

[1]*International Center of Excellence in Research (ICER-Mali), Filariasis Research and Training Unit, Bamako, Mali, Bamako, Mali,* [2]*International Center of Excellence in Research (ICER-Mali), Filariasis Research and Training Unit, Bamako, Mali/ Dermatology Hospital of Bamako, Bamako, Mali, Bamako, Mali,* [3]*International Center of Excellence in Research (ICER-Mali), Filariasis Research and Training Unit, Bamako, Mali/ Global Health Institute, Faculty of Medicine & Health Sciences, University of Antwerp, Antwerp, Belgium, Bamako, Mali,* [4]*Faculty of Medicine and Odonto-stomatogy/ University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali*

Schistosomiasis is affecting nearly 240 million people worldwide living mainly in sub-Saharan Africa. In Mali, the prevalence was 27.8% in 2007. In Kenieba health district (HD), the prevalence rose from 6.6% in 2007 to 78.5% in 2016 despite the yearly mass drug administration of praziquantel. Community members' knowledge level and involvement in control activities have been reported to be a crucial factor in program success. The current study aimed to assess the knowledge, attitudes and practices of school-age children and adults about urinary schistosomiasis. A cross-sectional study that covered 822 school-aged children (9-14 years) and adults (15-65 years) was conducted from June to August 2019 in Kenieba. A cluster sampling design was used to estimate the level of knowledge about schistosomiasis transmission, symptoms, prevention and control means in 30 randomly selected villages among the 240 villages. In each village, 30 households were randomly selected. All assenting/consenting children and adults were enrolled. Overall,

women 55.5% (456/822) were more represented than men with a sex ratio of 0.80. Within school-aged children, males were more represented 55.1% (86/156) while females were more represented among adults 58% (386/666). Participants who reported having schistosomiasis was comparable between children 38.5% (60/156) and adults 34.7% (231/666); p = 0.374. In both groups, most of the participants reported not knowing the modes of transmission of schistosomiasis (93.7% (624/666) for adults and 94.9% (148/156) for children; p = 0.579). Comparable number of adults and children (6.5% (43/666) and 5.1% (8/156)) reported being aware of schistosomiasis prevention methods; p = 0.750. Hematuria was recognized as a symptoms by more than 30.8% (253/822) of the participants but only 21.2% (141/666) of adults and 19.9% (31/156) of children were able to correctly state an additional symptom or sign. These data demonstrate a critically low level of knowledge of schistosomiasis in Kenieba. Tailored interventions should be validated and implemented to strengthen the level of knowledge of schistosomiasis in order to control this NTD.

419

DISTRIBUTION OF HYPER-ENDEMIC FOCI OF URINARY SCHISTOSOMIASIS IN MALI

Mahamadou Traore[1], Moussa Sangare[2], Yaya Ibrahim Coulibaly[3], Housseini Dolo[2], Salif Seriba Doumbia[2], Ilo Dicko[2], Abdoul Fatao Diabate[2], Lamine Soumaoro[2], Michel Emmanuel Coulibaly[2], Abdallah Amadou Diallo[2], Boubacar Guindo[4], Modibo Keita[4], Seydou Doumbia[2]

[1]*National Program for control and eliminate of Schistosomiasis and Soil-transmitted helminthes, Mali, Bamako, Mali,* [2]*International Center of Excellence in Research (ICER-Mali), Filariasis Research and Training Unit, Bamako, Mali, Bamako, Mali,* [3]*International Center of Excellence in Research (ICER-Mali), Filariasis Research and Training Unit, Bamako, Mali/ Dermatology Hospital of Bamako, Bamako, Mali, Bamako, Mali,* [4]*Hellen Keller International, Bamako, Mali*

Schistosomiasis is a widespread parasitic disease in tropical areas within poor communities. In Mali, its control activities are being implemented since several decades. They consist of preventive chemotherapy and sensitization for behavioral change. Nevertheless, urinary schistosomiasis continues to be endemic in the country. This study was designed to assess the distribution of hyper endemic foci of urinary schistosomiasis in Mali. It was a cross sectional study among school age children aged 7-14 years in 46 health districts classified into eco-climatic areas such as : Bamako the urban capital city, along the Niger River, along the Senegal River, Northern Sudan, Dogon Plateau, ponds in Sahel, Sudanese, flooded area. In each area, in each purposeful selected village, 60 school-aged children (SAC) were tested according WHO recommended guidelines, 30 of each gender. Ten ml of urine from each participant was collected, filtered using Whatman filter paper before staining with ninydrin 3% prior to a macroscopic examination on site to look for *Schistosoma haematobuim* eggs. Among the 6,489 SAC tested in 107 villages, the sex ratio was 1.07. About half of the study villages 44.85% (48/107) had low prevalence (between 0 and 10%) followed by moderate prevalence villages 34.57% (37/107) (between 10 and 50%) and high prevalence villages 20.6% (22/107) (above 50%). However, the prevalence of the disease was more common in men 25.7% (863/3357) than in women 20.4% (639/3132), $Chi^2 = 25.34$ $p<10^{-6}$. All the eco-climatic zones were found endemic for urinary schistosomiasis with prevalence ranging from 0% to 98.3%. However, the villages surrounding Bamako, the capital city 77.7% (7/9), the Sudanese zone 28.6% (6/21) and the Sahel ponds areas 23.5% (4/17) had the highest prevalence of schistosomiasis among the participants. Urinary schistosomiasis is still endemic in Mali with hyper-endemic foci whose prevalence are very high within SAC in Sudanese area, the villages near the Niger river surrounding the capital city. Hence, further investigations are needed to determine the role of young boys in the transmission of urinary schistosomiasis in endemic areas.

420

PREVALENCE AND FACTORS ASSOCIATED WITH SCHISTOSOMIASIS AMONG ADULTS AGED 18-35 YEARS IN KISUMU COUNTY, WESTERN KENYA

Valentine Sing'oei[1], John Owuoth[1], June Otieno[2], Chiaka Nwoga[3], Adam Yates[3], Ben Andagalu[2], Nathanial K. Copeland[2], Trevor A. Crowell[3], Christina Polyak[3]

[1]*HJF Medical Research International, Kisumu, Kenya /U.S. Army Medical Research Directorate – Africa, Kisumu, Kenya,* [2]*U.S. Army Medical Research Directorate – Africa, Kisumu, Kenya,* [3]*U.S. Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, MD, USA/ Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD, United States*

Schistosomiasis is a parasitic infection associated with significant morbidity and mortality and prevalent in tropical regions. It has been associated with increased susceptibility to HIV acquisition and adversely affects disease progression, making it a substantial public health threat in endemic areas. Understanding factors associated with schistosomiasis would inform the design and implementation of control programs. We assessed the prevalence of schistosomiasis in adults living with and at risk for HIV in Kombewa, Kenya. These cross-sectional analyses utilized data from men and women aged 18-35 years upon enrollment into a prospective cohort study from February 2017 through May 2018. Seroprevalence, reflecting prior pathogen exposure, was evaluated using an antibody test. Active schistosomiasis was diagnosed using circulating cathodic antigen (CCA) rapid tests on urine samples. Schistosomiasis prevalence was compared across demographic and other variables of interest using chi-square, T-test and Wilcoxon rank test as applicable. Of the 671 participants enrolled, 363 (54%) were male and 52 (8%) were living with HIV. Their mean age was 25.3 ± 4.3 (standard deviation [SD]) years and mean CD4 count was 838 ± 275 (SD) cells/mm^3. Schistosomiasis seroprevalence was 44% (274/620) and 66% (418/629) based on IgG and IgM, respectively. Active infection prevalence was 44% (296/669) based on CCA. Active schistosomiasis was more common among men (57% vs. 30%, p<0.0001), participants with less than secondary education (54% vs. 31%, p<0.0001), and participants living with HIV (58% vs. 43%, p=0.03). The high prevalence of schistosomiasis among adults in this study underscores the need to intensify control efforts, particularly among men, less-educated individuals, and those living with HIV.

421

PREVALENCE AND DISTRIBUTION OF SCHISTOSOMIASIS AND SOIL TRANSMITTED HELMINTHS IN 131 DISTRICTS OF 15 PROVINCES OF ANGOLA, 2018-2019

Elsa Mendes[1], Rilda Cristóvão[1], Maria Cecilia Almeida[1], Nzuzi Katondi[2], Ricardo Thomson[3], Sylvain Mupoyi[4], Onesime Ndayishimiye[5], Ferdinand Djerandouba[5], Mary Chimbili[5], Julio Ramirez[5], Sergio Lopes[5]

[1]*NTD Control Section, National Directorate for Public Health, Ministry of Health Angola, Luanda, Angola,* [2]*WHO Angola, Luanda, Angola,* [3]*WHO Afro Temporary Technical Advisor, Brazzavile, Republic of the Congo,* [4]*NTD Program, Ministry of Health, Kinshasa, Democratic Republic of the Congo,* [5]*The MENTOR Initiative, Luanda, Angola*

Schistosomiasis (SCH) and Soil-Transmitted Helminths (STH) are parasitic infections which can be controlled with mass drug administration strategies based on accurate disease prevalence data. The objective of this study is to determine the prevalence of SCH and STH in school-aged children in 15 provinces of Angola. The 15 provinces were mapped using WHO protocols; probability proportional to size (PPS) sampling was used to select a total of 640 schools and an average of 50 students per school (N=31.938 children). Faecal and urine samples were collected and processed using the Kato-Katz method and Urine Filtration. Prevalence estimates were calculated for each district (STH) and sub-district (SCH) with a 95% Confidence Interval. Bivariate and multivariate analysis was conducted to verify possible associations between infection rates and

other variables. Of the 131 districts surveyed, 112 (85.5%) are endemic for STH, 30 (22.9%) have a prevalence above 50%, 24 (18.3%) have a prevalence between 20% and 50%, and 58 (44.3%) have a prevalence below 20%; similarly, 118 (90,1%) of surveyed districted are endemic for SCH, 2 (1.5%) have a prevalence above 50%, 59 (45%) have a prevalence between 10% and 50%, and 57 (43.5%) have a prevalence below 10%. The districts with the highest STH prevalence are Bolongongo (94.5%), Banga (87.5%), and Kiwabi-Nzogi (84%). The sub-districts with the highest prevalence are Huila Sede (100%), Kahombo (92%), and Mupa (89%). There is a clear concentration of high STH infection rates in the northern provinces of Malanje and Lunda Norte, and high SCH infection rates in the southern provinces of Benguela and Huila. The results of the first national level SCH and STH mapping in Angola provide evidence to guide mass drug administration strategies in eligible areas for the next 5 years.

422

UROGENITAL SCHISTOSOMIASIS AMONG PRE-SCHOOL AND SCHOOL AGED CHILDREN IN FOUR DISTRICTS OF NORTHWESTERN TANZANIA AFTER A DECADE OF MASS DRUG ADMINISTRATION: GEOGRAPHICAL PREVALENCE, PERFORMANCE OF HAEMATURIA REAGENT STRIPS AND ASSOCIATED FACTORS

Humphrey D Mazigo[1], Cecilia Uisso[2], Upendo Mwingira[3], Paul Kazyoba[2], Maria Zinga[1], Safari M. Kinung'hi[4], Francesca Mutapi[5]

[1]*Department of Medical Parasitology, School of Medicine, Catholic University of Health and Allied Sciences, Mwanza, United Republic of Tanzania,* [2]*National Institute of Medical Research, Dar es Salaam, United Republic of Tanzania,* [3]*RTI, Washington, DC, United States,* [4]*National Institute of Medical Research, Mwanza, United Republic of Tanzania,* [5]*NIHR Global Health Research Unit Tackling Infections to Benefit Africa, University of Edinburgh, Ashworth Laboratories, King's Buildings, Edinburgh, United Kingdom*

After a decade of mass drug administration against *Schistosoma haematobium* in Tanzania, there is a need to assess the extent of disease burden in endemic districts, the performance of routinely used diagnostic tests and factors associated with infection to facilitates planning and implementation of intervention measures at local level. A total of 20,389 children from randomly selected 88 primary schools participated. A single urine sample was obtained from each participating child and examined visually for presence of macrohaematuria, with reagent strip test to detect microhaematuria and with urine filtration technique to detect and counting *S. haematobium* eggs. Overall, 7.4% (95%CI: 7.0-7.7, 1514/20,389) had *S. haematobium* egg-positive, with male having the highest prevalence (5.9% vs 9. %, *P*<0.001). The Geometrical Mean Eggs counts was 15.8eggs/10mls (95%CI: 14.8-16.8) of urine and male had the high eggs counts (t=-6.9256, *P*<0.001). Itilima district had the highest prevalence (12.7%, range:0-32%), while Nzega district had the lowest prevalence (1.1%, range: 0-4.1% at school level). The prevalence of microhaematuria was 9.3% (95%CI:8.9-9.7), the sensitivity and specificity of the urine reagent strip were 78% (95%CI: 76.1-79.9) and 99.8% (95%CI: 99.7-99.9) respectively. The odd of having microhaematuria positive results were associated with having light (*P*<0.001) and heavy infection intensities (*P*<0.001) and living in the study districts. Factors associated with *S.haematobium* infection were being male (aOR=1.59,95%CI:1.4-1.8,*P*<0.001), living in Shinyanga rural (aOR=4.8,95%CI:3.5-6.7,*P*<0.001), Bariadi (aOR=7.3,95%CI:5.6-9.6,*P*<0.001) and Itilima (aOR=13.2,95%CI:10.2-17.1,*P*<0.001). The findings provide an updated geographical prevalence which gives an insight on the planning and implementation of MDA. The urine reagent strips remain as a useful adjunct diagnostic test for rapid monitoring of urogenital schistosomiasis in areas with low and high prevalence. Based on prevalence levels, we recommend a timed and selective annual or after every one-year MDA in some schools.

423

ABO BLOOD GROUPS DO NOT PREDICT SCHISTOSOME INFECTION PROFILES IN HIGHLY ENDEMIC VILLAGES OF UGANDA

Rachel Francoeur[1], Moses Arinaitwe[2], Alon Atuhaire[2], Poppy Lamberton[1], Simon Babayan[1], Edridah Muheki[2]

[1]*University of Glasgow, Glasgow, United Kingdom,* [2]*Vector Control Division, Ministry of Health, Kampala, Uganda*

Schistosoma mansoni is a parasite which causes significant public health issues infecting over 220 million people globally. In Uganda alone approximately 11.6 million people are affected. Despite over a decade of mass drug administration in this country, hyperendemic 'hotspots' continue to persist. Blood type antigens play a known role in risk, resistance, and infection intensity for a variety of diseases due to cross-reactivity between host antibodies and pathogenic antigens. The aim of this study was to examine the effect of blood type as a potential intrinsic host factor that might be contributing to high levels of *S. mansoni* infections in hyperendemic areas of Uganda. To mitigate limitations in determining infection status, the study used longitudinal infection data to measure infection intensity patterns over time and to analyse associations with blood type. Other biometric parameters including age, gender, and BMI have previously been established as significant variables influencing the prevalence and intensity of schistosomiasis. A mixed general linear regression model was used to evaluate correlations between blood type, age, gender, BMI and variance among three different villages. The model revealed no associations between blood type and infection intensity. Variations in infection profiles were significantly different between the villages and egg burden significantly decreased with age. While blood type has proven to be a predictor of numerous diseases, the data collected in this project indicate that it does not play a role in predicting *S. mansoni* infection status.

424

DEVELOPMENT OF COMMUNITY EFFORTS TOWARDS SCHISTOSOMIASIS VECTOR GENETIC TOOLS AND CONTROL STRATEGIES

Nicolas J. Wheeler[1], Nathalie Dinguirard[1], Jutta Reinhard-Rupp[2], David Rollinson[3], Timothy P. Yoshino[1], Mostafa Zamanian[1]

[1]*University of Wisconsin-Madison, Madison, WI, United States,* [2]*Merck KGaA Global Health Institute, Eysins, Switzerland,* [3]*Natural History Museum, London, United Kingdom*

Transmission of vector-borne parasites with indirect life cycles relies upon the infection of an intermediate host, forming a bottleneck for disease propagation. Vector-control for the interruption of disease transmission has long been a consideration for parasitic diseases like malaria and schistosomiasis, but the concept has recently taken center stage with the development of genome editing tools that may enable techniques for introducing gene drives to suppress or replace susceptible vector populations. The protocols for generating modified organisms are highly developed for many mosquito species that vector the etiological agents for malaria, Dengue, lymphatic filariasis, and other diseases, and modified mosquitoes are actively being released into the wild in some endemic communities. However, similar protocols have lagged for the aquatic pulmonate snails that vector schistosomiasis. We have begun developing these tools for the model schistosome vector *Biomphalaria glabrata* and have focused first on optimizing them in the *B. glabrata* embryonic cell line. First, we report the ability to deliver fluorescent reporters via transfection or electroporation at efficiencies of ~15% to ~50%, respectively. Attempts to edit the genome with CRISPR/Cas9 are ongoing and are hindered by the mosaicism, nucleotide variation, and immense aneuploidy of Bge strains. Second, we report the establishment of techniques to deliver materials to the early embryo of *B. glabrata* and raise them to the juvenile stage via *in vitro* culture. Finally, we announce the establishment of a workstream for snail research and tool development,

organized by the Global Schistosomiasis Alliance. We anticipate that this public consortium will provide a foundation for essential global health discussions and will scaffold future vector-control research collaborations.

425

MATERNAL SCHISTOSOMIASIS SYSTEMICALLY MODULATES ADAPTIVE IMMUNITY

Lisa C. Gibbs, Diana Cortes-Selva, Atakan Ekiz, Keke Fairfax

University of Utah, Salt Lake City, UT, United States

Maternal helminth infections are a global public health concern that correlate with altered infant immune responses to childhood immunizations. A mechanistic understanding of how maternal helminth infection alters the cellular immune responses of offspring is lacking, but is critical to improve childhood vaccine regimens in endemic areas and to understand the consequences of specific long-lived immunity defects. Children born to mothers infected with Schistosomiasis often have measurable Schistosome egg antigen (SEA) titers that correlate with maternal titers. Using our newly established model of model of maternal *Schistosoma mansoni* infection in dual IL-4 reporter mice, we find that murine pups born to mothers chronically infected with *Schistosoma mansoni* have anti-SEA titers that correlate with maternal titers, similar to what has been reported in human maternal infections. Additionally, offspring of infected mothers have reduced peripheral memory B and Th2 cells at steady state and following Tetanus/Diphtheria immunization. To determine the transcriptomic mechanism that may underlie this defect, we used a single cell sequencing approach on age matched pups born to chronically *S. mansoni* infected mothers, mothers infected with a single sex infection, and uninfected mothers. We have identified an egg-dependent pattern of transcriptional alterations to B and T cells that center on control of cell cycle and proliferation. We hypothesize that this transcriptional profile is the mechanistic root of long-lived defects in cellular immunity to foreign antigens.

426

EVALUATION OF CRASSPHAGE MARKER FOR TRACKING FECAL CONTAMINATION IN THE BAGMATI RIVER, NEPAL

Lauren M. Ward[1], Rajani G. Shrestha[2], Sarmila Tandukar[3], Jeevan B. Sherchand[4], Eiji Haramoto[2], Samendra P. Sherchan[1]

[1]*Department of Environmental Health Sciences, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States,* [2]*Interdisciplinary Center for River Basin Environment, University of Yamanashi, Kofu, Japan,* [3]*Department of Natural, Biotic and Social Environmental Engineering, University of Yamanashi, Kofu, Japan,* [4]*Institute of Medicine, Tribhuvan University, Kathmandu, Nepal*

Enteric viruses in the aquatic environment are a concern due to the potential for waterborne disease transmission to humans. In Nepal, the Bagmati River serves as a source of drinking, and irrigation water, so the detection of waterborne enteric pathogens is integral to maintaining human health. The objective of this study was to quantify the crAssphage marker in surface water samples from the Bagmati River between November 2015 and September 2016. Concentrations of crAssphage were then compared to those of other enteric viruses and indicator organisms found in the samples in order to examine the potential of crAssphage as a marker for fecal contamination. CrAssphage was detected in 17% (1/6) of samples from Sundarijal, 100% (6/6) of samples from Thapathali, and 100% (6/6) samples from Chovar, with the highest average concentrations recorded in May 2016 and the lowest average concentrations recorded in September 2016. Overall, crAssphage was present in 72% (13/18) of samples and was strongly correlated with the presence of fecal indicator bacteria E. coli (r= 0.89) and Enterococcus (r = 0.93) and several enteric viruses. The strongest viral correlations were to salivirus (r = 0.84), pepper mild mottle virus (r = 0.76), Aichi virus 1 (r = 0.75), enterovirus (r = 0.61), and tobacco mosaic virus (r = 0.7138). These results provide evidence for the potential use of crAssphage as a marker for human fecal contamination in the Bagmati River.

428

BACTERIAL AND PARASITIC CONTAMINATION OF SATCHET WATER BRANDS SOLD AT NGWO, ENUGU STATE NIGERIA

Pauline U. Umeanaeto[1], Ezinne G. Ani[1], Victoria I. Anyaoha[1], Miriam O. Iwueze[1], Chukwudinma C. Okoli[2]

[1]*Nnamdi Azikiwe University, Awka, Anambra State, Nigeria,* [2]*Hospital Management Board, Laboratory Unit, Kuje General Hospital,, Federal Capital Territory, Abuja, Nigeria*

Water is an essential part of human nutrition. Unsafe drinking water is a key determinant of many bacterial and parasitic diseases with serious complication on human health globally. This study focuses on isolation and identification of bacteria and parasites contaminating different brands of sachet water sold at Ngwo, Enugu State, Nigeria. Twenty one sachets of water were collected randomly from seven different brands. The parasites were identified using sedimentation technique while bacteria were isolated from water cultured in Nutrient, MacConkey and Eosine Methylene Blue Agar media. The physicochemical parameters analyzed were: total dissolved solids, pH, chloride, total hardness and nitrite. Two-way analysis of variance was employed to analyze the data for bacteria and parasites identified. Of the 21 sachet water samples examined, 13(61.9%) were positive with parasites. Two parasite species [Cysts of Entamoeba histolytica 4(19.0%) and Giardia lamblia 9(42.8%)] were identified. The total viable bacteria count ranged from 4(6.5%) to 10(16.1%) colonies in 1ml of water whereas the total Coliform count ranged from 4(6.5%) to 6(9.7%) in 1ml of water with sample F having the highest value of both total viable bacterial count and total Coliform count. The percentage of the total viable bacterial count recorded was 75.8% while the total Coliform count was 24.2%. There was significant difference in the distribution of bacteria among different brands of water samples ($p<0.05$). Bacillus sp 10(29.4%) had the highest occurrence whereas Micrococcus sp 3(8.8%) had the least which was not significant ($P>0.05$). The physicochemical parameters fall within the recommended limit set by World Health Organization for drinking water except the pH value of two brands which was below the stipulated limit. The presence of coliform and other microbes in the samples could have unhealthy implications in consumers when consumed. There is need for regular and periodic monitoring of the water quality before and after production.

1

429

REDUCING DISABILITY ADJUSTED LIFE YEARS (DALYS) LOST AMONG CLIMATE REFUGEES IN DHAKA

Neelima Afroz Molla[1], Kabirul Ahsan Mollah[2], Ghaffar Ali[3], Oleg Shipin[4], Pongrama Ramasoota[5]

[1]*BCSIR, Dhaka, Bangladesh,* [2]*Oxford College of Arts, Business & Technology, Toronto, ON, Canada,* [3]*University of Agriculture Faisalabad, Faisalabad, Pakistan,* [4]*Asian Institute of Technology, Pathumthani, Thailand,* [5]*Mahidol University, Bangkok, Thailand*

Climate change the cruel reality that Bangladesh faces relentlessly in particular way of human migration or displacement and made inland-refugee. They are poor, they are the climate refugees - together in one identity pushes them to endure double effects of climate change. Climate change's callous effects force this people to become refugees and then local climate pose a high threat to their health (e.g intestinal health condition specially diarrhoea). Diarrhoea is a climatic parameters sensitive tropical infectious disease amongst the most vulnerable group poor children. This disease is more prevalent and one of the major causes of child morbidity & mortality among them. In this study we focused on household level socio-environmental factors that used for disease burden quantification. Moreover, considering local level climatic variables (temperature, humidity and rainfall) coupled with increased Disability Adjusted Life Years (DALYs) lost due to diarrhea in refugee children. We

used population-based holistic approach SEE (Socio-epidemiological and environmental) for quantification of health losses. Based on diarrhoea morbidity and mortality data, DALYs lost was calculated. An estimated multifold (14 folds/child/household) DALYs lost in climate refugee communities due to diarrhea compare to non-climate refugee. 79% diarrhea burden was increased with temperature variations. The strength of associations is estimated by population average models using Generalized Estimating Equations (GEE) approach, where DALYs showed highly significant relations (p >0.001) with changes Odds Ratio (OR) ranges from 4.6 - 7.9 for some selected predictors. Influencing factors, such as household level water treatment, shared sanitation practice, hygiene behaviors such as children open defecation habit, and maternal literacy strongly attenuated DALYs lost. Local policy maker should consider climatic variables when safeguarding children's health for developing climate change adaptation and pediatric care policies.

430

AIRBORNE FUNGI SPORE DISTRIBUTION IN TWO HOSPITALS IN KABALE DISTRICT, SOUTHWEST, UGANDA

Adeyinka Odebode, Gerald Niwamanya

Kabale University, Kabale, Uganda

Fungi are an increasing public health problem worldwide. Fungi particles also induce an allergic response in susceptible individuals. Hospital environments contain different types of microorganisms such as airborne fungi which causes fungal diseases. In the present study, the total count and diversity of airborne filamentous and yeasts fungi were investigated in indoor air of selected wards of two major hospitals in Uganda. Samples of indoor air from Outpatient ward, Maternity, Pediatrics and Emergency wards were collected by open plate technique on Potato dextrose agar media once a week. Samples were collected in triplicates. The study also examined the proportion in fungal infection cases most commonly reported in the two hospitals. The cultures were then examined and evaluated according to macroscopic and microscopic examination criteria for genotypic identifications. The obtained results were analyzed by SAS and Plotly software. In this study, a total of 22 different fungi species were isolated from the two hospitals with *Aspergillus flavus* (17.9%) followed by *Aspergillus fumigatus* (12.3), *yeast* (9.6), *penicillium citrinum* (8.5%), as the most abundant and frequently surveyed fungal species while *Trichoderma*, *Nigrospora* and *P. marneffei* had the least values of spore count. All the wards showed high rates of contamination by various fungi. However, the analysis of the data showed that indoor air of OPD (28.4%) had the highest number of fungi colonies in Kabale hospital while maternity ward (31.1%) had the highest for Rugarama hospital. Females also had more asthma cases in both months for Kabale hospital with patient's ages 6-59 years visiting the hospital for either asthma or fungi infection cases while for Rugarama hospital, fungi infection cases were more prevalent than asthma cases. SDS-PAGE analysis revealed a total of 21 protein bands with molecular weight between 5 and 100 kDa. Rainfall and relative humidity were positively correlated with high fungi load in the atmosphere of the two hospitals. Data on the abundance/prevalence of fungi spores in hospital environment of sub-Saharan Africa is limited.

431

ASSESSING BEHAVIOUR TOWARDS THE UPTAKE OF A NOVEL LOW-COST WATER FILTRATION SYSTEM IN SCHISTOSOMIASIS-ENDEMIC COMMUNITIES IN MWANZA, TANZANIA

May N. Sule[1], Safari M. Kinung'hi[2], Askwar Hilonga[3], Justina Mosha[2], Michael R. Templeton[1]

[1]*Imperial College London, London, United Kingdom,* [2]*National Institute for Medical Research, Mwanza Centre, Mwanza, United Republic of Tanzania,* [3]*The Nelson Mandela African Institution of Science and Technology, Arusha, United Republic of Tanzania*

This pilot study aimed to develop an evidence base around behaviour towards the uptake of a novel low-cost water filtration system, Nanofilter®, to address household water supply in schistosomiasis-endemic communities presently not effectively served by other water supply infrastructure. The study is assessing the willingness and experiences of using the water system in the community. The pilot is located in three communities, Kigongo, Bukumbi and Mwasonge, around Lake Victoria in Mwanza region, Tanzania. The study has applied a three-step participatory approach: firstly, the Nanofilter® equipment is installed in water stations and local water supervisors trained in operation and maintenance of the system; Secondly, monitoring of the water stations and measuring water output with the data used to assess consumption; and thirdly, using quantitative and qualitative methods alongside observations to assess behaviour and uptake, as well as determining the technical, social and cultural challenges and barriers to address for long term sustainability of the Nanofilter® water treatment system. The initial acceptance of the water systems by the community has been very positive considering that they are paying for this alternative safe water. Quantitative and qualitative end-point surveys later in the year (August 2020) will provide data on the sustained uptake and impact that the water stations are having on community drinking water demand and will quantify other benefits realised from the water systems. The results and lessons learnt will provide valuable knowledge on addressing gaps in critical knowledge on behaviours and attitudes that could prevent uptake, as well as practical guidance for effective and acceptable socio-economic strategies to improve water uptake in schistosomiasis-endemic communities. The conclusions from this study will lead to effective and sustainable ways of inserting technology and provide good insight for many future water technology insertion projects that will help reduce transmission of schistosomiasis and other waterborne diseases.

432

NON-CHOLERA INVASIVE VIBRIOSIS RATES IN MARYLAND ARE RELATED TO YEARLY WATER TEMPERATURES

Naomi Hauser[1], Amanda Thepotte[1], Gregory Schrank[2], Ronald Rabinowitz[2]

[1]*University of Maryland Medical Center, Baltimore, MD, United States,* [2]*University of Maryland School of Medicine, Baltimore, MD, United States*

Over the last century, global ocean temperatures have risen by an average of 1.0°C according to the National Oceanic and Atmospheric Administration (NOAA), with large annual temperature fluctuations of up to 0.28°C. Changing temperatures have a significant impact on global climate events, as well as on the rates of waterborne infections. Globally, there have been an increasing number of outbreaks of non-cholera vibrio infections associated with increases in seawater temperatures due to climate change. We became interested in the relationship between statewide non-cholera invasive vibriosis cases in Maryland and local water surface temperatures in recent years. We obtained monthly non-cholera vibriosis rates from the Maryland Department of Health for the years 2010-2017. The data comprise non-cholera *Vibrio* species detected from all sites other than stool, including blood, wound/tissue, sputum, ear drainage, and urine. Water temperature data is from the Baltimore Chesapeake Bay site and is publicly available from NOAA. Invasive vibriosis rates occur during the warmer months with higher surface water temperatures and a statistically significant correlation between temperature and incidence (Spearman's coefficient 0.708, $p<0.001$. Although no statistically significant increase in non-cholera invasive vibriosis rates could be identified (Spearman's coefficient 0.452, $p=0.260$), there are noticeable peaks in 2012 and 2016 corresponding to years with remarkably high surface water temperatures both in our study (2012) and on a global average (2016). Our data contribute to the growing body of reports of increased vibriosis incidence in the setting of warmer water temperatures. Although our data was not statistically significant, there is evidence of increased vibriosis rates during years with warmer Chesapeake Bay and global water temperatures which is remarkable on a time scale of only 8 years.

433

EVALUATION OF A SOCIAL MARKETING PROGRAM ON ACCESS TO HEALTH PRODUCTS IN KENYA, 2014-2016

Sunkyung Kim[1], Mark Laughlin[1], Ronald Otieno[2], Aloyce Odhiambo[2], Jared Oremo[2], Jay Graham[3], Mitsuaki Hirai[1], Emma Wells[1], Colin Basler[1], Anna Okello[2], Almea Matanock[1], Alie Eleveld[2], Rob Quick[1]

[1]*Centers for Diase Control and Prevention, Atlanta, GA, United States,* [2]*Safe Water and AIDS Project, Kisumu, Kenya,* [3]*University of California, Berkeley, CA, United States*

The Safe Water and AIDS Project (SWAP), a non-governmental organization in western Kenya, opened kiosks run as businesses by community health promoters (CHPs) to increase access to health products among poor rural families. We conducted a baseline survey in 2014 before kiosks opened, and a post-intervention follow-up in 2016, enrolling 1,517 households with children <18 months old. From baseline to follow-up, we observed increases in reported exposure to the SWAP program (3% to 11%, p=0.01), and reported purchases of any SWAP product (3% to 10%, p<0.01). The percent of households with confirmed water treatment (detectable free chlorine residual [FCR] >0.2 mg/ml) was similar from baseline to follow-up (7% vs. 8%, p=0.57). The odds of reported diarrhea in children decreased from baseline to follow-up (odds ratios or OR: 0.77, 95% CI: 0.64-0.93) and households with detectable FCR had lower odds of diarrhea (OR:0.53, 95% CI:0.34-0.83). Focus group discussions with CHPs suggested that high product prices, lack of affordability, and expectations that products should be free contributed to low sales. In conclusion, modest reported increases in SWAP exposure and product sales in the target population were insufficient to impact health, but children in households confirmed to chlorinate their water had decreased diarrhea.

434

EFFECT OF A WATER, SANITATION, AND HYGIENE PROGRAM ON HANDWASHING WITH SOAP AMONG HOUSEHOLD MEMBERS OF DIARRHEA PATIENTS IN HEALTH FACILITIES IN BANGLADESH: A CLUSTER-RANDOMIZED CONTROLLED TRIAL OF THE CHOBI7 MOBILE HEALTH PROGRAM

Fatema Zohura[1], Md. Sazzadul Islam Bhuyian[1], Ronald Saxton[2], Ronald Saxton[2], Tahmina Parvin[1], Shirajum Monira[1], Shirajum Monira[1], Shwapon Kumar Biswas[1], Jahed Masud[1], Sharika Nuzhat[1], Nowshin Papri[1], M Tasdik Hasan[1], Elizabeth Thomas[3], David A. Sack[3], Jamie Perin[3], Munirul Alam[1], **Christine Marie George**[3]

[1]*International Center for Diarrheal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh,* [2]*Johns Hopkins School of Public Health, Baltimore, MD, United States,* [3]*Johns Hopkins University, Baltimore, MD, United States*

The Cholera-Hospital-Based-Intervention-for-7-days (CHoBI7) is a water treatment and handwashing with soap intervention for patients and household members which is initially delivered in a health facility setting. This study evaluated the effectiveness of CHoBI7 program delivery in increasing handwashing with soap in a health facility setting. A randomized controlled trial of the CHoBI7 program was conducted among 404 diarrhea patients and their accompanying household members in health facilities in Dhaka, Bangladesh. The "Standard Message" arm received the standard message given in Bangladesh to diarrhea patients on the use of oral rehydration solution. The "Health Facility Visit + Soapy Water" arm received the standard message, the CHoBI7 communication module delivered bedside to the patient; and a soapy water bottle in the health facility. The "Health Facility Visit + Handwashing Station" arm received this same intervention plus a small plastic handwashing station. Within 24 hours of intervention delivery, three-hour structured observation of handwashing practices at stool/vomit and food related events (key events) was conducted in health facilities. Compared to the Standard Message Arm, there was significantly higher handwashing with soap at key events in both the Health Facility Visit + Handwashing Station Arm (58% vs. 25%) (Odds Ratio (OR): 4.12; (95% Confidence Interval (CI): 1.86, 9.14), and the Health Facility Visit + Soapy Water Arm (51% vs. 25 %) (OR: 3.02; (95% CI: 1.41, 6.45). These findings demonstrate that delivery of the CHoBI7 module presents a promising approach to increase handwashing with soap in a health facility setting in Bangladesh.

435

PROCESS EVALUATION FOR THE DELIVERY OF A WATER, SANITATION, AND HYGIENE MOBILE HEALTH PROGRAM: FINDINGS FROM THE RANDOMIZED CONTROLLED TRIAL OF THE CHOBI7 MOBILE HEALTH PROGRAM

Md. Sazzadul Islam Bhuyian[1], Ronald Saxton[2], Khaled Hasan[2], Jahed Masud[1], Fatema Zohura[1], Shirajum Monira[1], Shwapon Kumar Biswas[1], M Tasdik Hasan[1], Tahmina Parvin[1], Ismat Minhaj Uddin[1], Kazi Md. Zillur Rahman[3], Nowshin Papri[1], Mahamud-ur Rashid[4], Lubaba Sharin[1], Alana Teman[2], Elizabeth D. Thomas[2], Kelsey Alland[2], Alain Labrique[2], David A. Sack[2], Jamie Perin[2], Munirul Alam[1], **Christine Marie George**[2]

[1]*International Center for Diarrheal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh,* [2]*Johns Hopkins University, Baltimore, MD, United States,* [3]*University of New South Wales, Sydney, Australia,* [4]*University of Manitoba, Winnipeg, MB, Canada*

The Cholera-Hospital-Based-Intervention-for-7-days (CHoBI7) mobile health (mHealth) program delivers mobile messages to diarrhea patient households promoting water treatment and handwashing with soap. The randomized controlled trial (RCT) of the CHoBI7 mHealth program demonstrated this intervention was effective in significantly reducing diarrhea and stunting in young children. The objective of this study was to assess the implementation of the CHoBI7 mHealth program in delivering mHealth messages during this RCT. Five-hundred seventeen diarrhea patient households received weekly text, voice, and interactive voice response (IVR) messages from the CHoBI7 mHealth program over the 12 month program period. The program process evaluation indicators were the following: the percentage of CHoBI7 mHealth messages received (program fidelity and dose) and fully listened to by program households (program fidelity and dose), and beneficiaries reporting receiving and sharing a mHealth message from the program (program reach) in the past two weeks. Ninety-two percent of text messages were received by program households. Eighty-three percent of voice and 86% of IVR messages sent were fully listened to by at least one household member. Eighty-one percent of IVR quiz responses from households were answered correctly. Program households reported receiving a CHoBI7 mHealth message in the past two weeks at 79% of monthly household visits during the 12-month program. Seventy-seven percent of participants reported sharing a program message with a spouse, 55% with a neighbor, and 49% with a child during the program period. There was high fidelity, dose, and reach of messages delivered for the CHoBI7 mHealth program. This study presents an approach for process evaluation that can be implemented to evaluate future mHealth programs.

436

DIARRHEAL DISEASE KNOWLEDGE AMONG HOUSEHOLD MEMBERS OF DIARRHEA PATIENTS: FINDINGS FROM THE RANDOMIZED CONTROLLED TRIAL OF THE CHOLERA-HOSPITAL-BASED-INTERVENTION-FOR-7 DAYS (CHOBI7) MOBILE HEALTH PROGRAM

Jahed Masud[1], Shwapon Kumar Biswas[1], Fatema Zohura[1], Md. Sazzadul Islam Bhuyian[1], Nowshin Papri[1], Fahmida Dil Farzana[1], Tahmina Parvin[1], Shirajum Monira[1], Munirul Alam[1], **Christine Marie George**[2]

[1]*International Center for Diarrheal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh,* [2]*Johns Hopkins University, Baltimore, MD, United States*

The objective of this study was to evaluate the impact of the Cholera-Hospital-Based-Intervention-for-7-days (CHoBI7) handwashing with soap

and water treatment mobile health (mHealth) program on diarrheal disease knowledge among diarrhea patients and their household members in urban Dhaka, Bangladesh. A cluster-randomized controlled trial of the CHoBI7 mHealth program was conducted among diarrhea patient households in Dhaka, Bangladesh. Patients were randomized to three arms: standard recommendation on oral rehydration solution use; health facility delivery of CHoBI7 plus mHealth (no home visits); and health facility delivery of CHoBI7 plus two home visits and mHealth. An open ended questionnaire was administered to 1468 participants 12 years of age or older on diarrheal disease transmission and prevention. These items were combined to form a diarrheal disease knowledge score measured at baseline and 1 week, 6 months, and 12 months. At baseline, when participants were asked to report three ways diarrheal diseases were spread 37% (546/1468) of participants reported by water, 13% (187/1468) reported by lack of handwashing, and 4% (53/1468) by food not being covered properly. At baseline when asked to name three ways diarrheal diseases could be prevented, 35% (515/1468) of participants reported safe water, and 16% (228/1468) reported handwashing with soap. Participants in both mHealth arms had significantly higher diarrheal disease knowledge scores at the 1 week, 6 month, and 12 month time points compared to the standard recommendation arm.These findings suggest the CHoBI7 mHealth program presents a promising approach to increase diarrheal disease knowledge among diarrhea patient households.

437

HEALTH BEHAVIORS AND THE SPATIAL DISTRIBUTION OF INTESTINAL PARASITE INFECTIONS IN SOUTH AMERICA

Nelson Atehortua De la Pena[1], Yohana Sarria-Guzman[2]

[1]*Jackson State University, Jackson, MS, United States,* [2]*Public Health Consultant, Cartagena, Colombia*

Background: Intestinal Parasitic Infections (IPI) are endemic in multiple areas of Latin America. They are associated with inadequate sanitation and water treatment systems, gaps in population's health knowledge, attitudes and practices (KAP); and poor health literacy. Geographic Information Systems (GIS) make possible to layout the distribution of IPI cases. COVID-19 has highlighted the need for improving and increasing hygiene, health promotion and education (HPE) interventions. IPI and GIS can work as proxy to measure improvements in sanitary infrastructure and behavioral risk factors. Aim: To describe the association between health behaviors and the spatial distribution of IPI in South America (SA). Method: A systematic review of the literature published in English within the last 10 years using the Matrix Method and the PRISMA statement guidelines was done on several curated electronic databases including, not limited to, Pubmed, Web of Science, Scopus, and Scielo. Only studies conducted in SA were included. Discrepancies in the analysis of were solved by consensus. Results: A total of seven studies including urban and rural sites were analyzed. By country, four studies were done in in Brazil, two in Colombia, and one in Argentina. The technique most frequently used for the identification of parasites was the Kato-Katz method. Multiple types of intestinal helminths and protozoan parasites were included in the studies. Regarding KAP, there was a lot of variability in the range of variables studied. None of the studies included map layers of existing local infrastructure nor made a description of the conditions of sanitation or the public home drinking water systems. Conclusions and recommendations: An important limitation of this study was that only a very small fraction of all the articles reviewed comply with the inclusion criteria. There is a need to increase the number and efficacy of HPE interventions, especially involving novel GIS technologies. Also, for comprehensive research including IPI, GIS and HEP interventions; now that COVID is circulating profusely and no safe and effective vaccine is available.

438

ASSOCIATIONS BETWEEN 8 EARTH OBSERVATION-DERIVED CLIMATE VARIABLES AND PATHOGEN-SPECIFIC ENTERIC INFECTIONS IN MULTIPLE LARGE SURVEILLANCE STUDIES

Josh M. Colston

University of Virginia School of Medicine, Charlottesville, VA, United States

Climate change threatens to undermine progress in reducing global childhood diarrheal disease deaths. However, lack of evidence of how individual environmental factors impact transmission of specific diarrheal pathogens makes predicting trends under different climate scenarios challenging. Research is needed to characterize associations between multiple meteorological exposures and individual enteric pathogens but until recently, accessing accurate and complete data on hydrometeorological predictors at high temporal resolution was difficult. Earth Observation (EO) climate data derived from satellites and model-based reanalysis are increasing in availability and accuracy and show the potential to address these knowledge gaps. Meanwhile, improved diagnostic methods, and several ambitious multi-site, population-based studies are shedding light on pathogen-specific etiologies of diarrheal disease. Under a NASA-funded collaboration, data from multiple multi-site studies have been compiled into a dataset consisting of results from around 60,000 stool samples from 17,000 infants in 24 different locations around the world, which were tested using quantitative polymerase chain reaction (qPCR) for infection status with 15 high-burden enteric pathogens - 5 viruses, 8 bacteria and 2 protozoa. Samples were matched by date and location to EO-derived estimates of hydrometeorological parameters extracted from the GLDAS global model. Generalized linear models were fitted to model associations with specific enteric pathogens adjusting for confounders. Complex, non-linear, species- and syndrome-specific associations were observed for all pathogens. For example, on days with high surface runoff, the risk of a subjects' stool being positive for rotavirus increased by 239%, while the equivalent risk of *Campylobacter* infection decreased by 30% relative to days with no surface runoff. Results like these are being used to create high resolution maps of pathogen-specific enteric disease transmission risk, which will be made available through an online platform for use in programmatic and policy decision-making.

439

MICROBIOME OF DOMESTIC WATER FROM RURAL COMMUNITIES IN THE SOUTHERN CARIBBEAN, WATER QUALITY AND HUMAN HEALTH IMPLICATIONS

Akilah T. Stewart[1], Adrian Cashman[2], Dave Duman Chadee[1], Adesh Ramsubhag[1]

[1]*The University of the West Indies, St. Augustine, Trinidad and Tobago,* [2]*The University of the West Indies, Cavehill Campus, Barbados*

The microbiological quality of drinking water is of primary importance for human health especially in tropical regions. The paradigm shift from traditional concepts of assessing water quality by detecting faecal contamination as a predictor of risk of enteric pathogenic exposure, to, a wider assessment of the impact of other microbes on human health overlaps with the rapidly evolving field of next generation sequencing. Characterizing the microbiome of domestic water using molecular tools has proven highly relevant in assessing human health risk. However, no studies exist on molecular microbial water quality in the Caribbean. This study seeks to characterize the bacterial community structure and identify organisms of health significance in domestic water from three rural communities in the Southern Caribbean using next-generation 16S rRNA amplicon sequencing. 318 samples were collected from Nariva, Trinidad (102), Carriacou, Grenada (94) and Speightstown, Barbados (119) during two sampling campaigns in the wet and dry season for each country, making it the largest drinking water study in the region. A total of 31708 operational taxanomic units (OTU) were included in analysis using Phyloseq, and identified from 26 phyla. A total of 3363 OTU's had an abundance of one hundred and more. The MDS ordination plot for communities showed that Carriacou and Nariva samples were most closely

related which shows some microbial community structure similarity across islands. Numerous genera of human health importance identified by WHO were found across all countries with many sequences of 100% match to the species level including for *Mycobacterium*, *Legionella* and *Aeromonas*. While *Escherichia* was detected, its relative abundance was far lower than these other genera highlighting the importance of alternative microbial water quality indicators in the tropics. PCR validation of genera of human health importance is on-going for these selected genera. These results would assist in informing water treatment protocols and guidelines, especially in rural communities where water harvesting and the use of other sources of environmental water is practiced.

440

INCREASING ACCESS TO MALARIA IN PREGNANCY SERVICES THROUGH COMMUNITY HEALTH UNITS AND ENHANCED SUPPORTIVE SUPERVISION OF COMMUNITY HEALTH VOLUNTEERS

Donald Apat[1], Willis Akhwale[1], Moses Kidi[1], Edwin Onyango[2], James Andati[1], Hellen Gatakaa[1], Augustine Ngindu[1], Lolade Oseni[3], Gladys Tetteh[3], Daniel Wacira[4]

[1]*PMI-Impact Malaria, Nairobi, Kenya,* [2]*Department of Health, Busia County, Kenya,* [3]*Jhpiego, Baltimore, MD, United States,* [4]*PMI, Nairobi, Kenya*

According to the 2018 Kenya malaria program review, the uptake of malaria in pregnancy interventions by rural communities in Kenya remains low due to late first presentation to antenatal care (ANC), leading to sub-optimal intermittent preventive treatment in pregnancy (IPTp) coverage. Poor healthcare provider-client communication and low investment in advocacy, communication, and social mobilization contribute to late ANC presentation. Kenya is using community health volunteers (CHVs) supervised by community health assistants (CHAs) in community health units (CHUs) to increase demand for ANC services and uptake of IPTp but tracking of progress is hampered by a lack of accurate data on the number of estimated pregnancies at the sub-national level and poor household coverage by CHVs at the community level. In July 2019, Impact Malaria supported malaria-endemic Teso South sub-county of Busia county with the reorientation of 354 CHVs (92%) and 14 CHAs (100%), to identify and track pregnant women at the household level within the government established CHUs, provide social and behavior change communication messages, and enhance monthly supervision and reporting by CHAs. CHVs identified and tracked 917 pregnant women from 32,758 (89.6%) households and identified and referred 273 ANC defaulters. We compared the uptake of IPTp before intervention (January to June 2019) and during the intervention (July to December 2019) using programmatic and Kenya health information system (KHIS) data. At pre-intervention, 32,898 (90%) households were visited, with 2,160 new ANC visits and 5,342 ANC revisits. During the intervention period, 35,910 (98.3%) households were visited with 1,934 new ANC visits and 5,904 ANC revisits. Uptake of IPTp1 increased from 83.6% to 92.6%; IPTp2 from 73.5% to 87%; and IPTp3 from 51.9% to 75.4%. Enhanced supervision of CHVs by CHAs to conduct and improve household visits enabled identification and referral of ANC defaulters and contributed to increased IPTp uptake. Supportive supervision and optimal CHU coverage in tracking pregnant women if conducted routinely may provide accurate denominators to track IPTp coverage and inform targeted interventions.

441

CO-IMPLEMENTING VITAMIN A SUPPLEMENTATION WITH SEASONAL MALARIA CHEMOPREVENTION IN SOKOTO STATE, NIGERIA: A FEASIBILITY STUDY

Olusola B. Oresanya[1], Abraham Ahmadu[1], Abimbola Phillips[1], Taiwo Ibinaiye[1], Olatunde Adesoro[1], Jamilu I. Nikau[2], Chris Isokpunwu[3], Rilwan M. Anka[4], Shiwan H. Dlakwa[4], Nana A. Ibrahim[4], Obinna Emeruwa[4], Ochogbu Paul[4], Abdulrahman A. Ahmed[5], Mohammad A. Inname[5], Charlotte Ward[6], Kevin Baker[6], Madeleine Marasciulo[7], Christian Rassi[6], Kolawole Maxwell[1], Helen Smith[6]

[1]*Malaria Consortium, Abuja, Nigeria,* [2]*National Malaria Elimination Programme, Abuja, Nigeria,* [3]*Federal Ministry of Health, Abuja, Nigeria,* [4]*Malaria Consortium, Sokoto, Nigeria,* [5]*Sokoto State Ministry of Health, Abuja, Nigeria,* [6]*Malaria Consortium, London, United Kingdom,* [7]*Malaria Consortium, North Carolina, NC, United States*

Of two billion people estimated to have micronutrient deficiencies globally, worst hit are children living in low and middle-income countries. Bi-annual high dose vitamin A supplements given to children aged 6-59 months can reduce all-cause mortality by 24%. Despite this, in 2018 vitamin A coverage was just 41% in Nigeria. WHO recommends vitamin A supplementation be integrated into other public health programmes aimed at improving child survival. Seasonal malaria chemoprevention (SMC), another high impact campaign-based intervention for children under five, provides a ready platform for improving Vitamin A coverage. Using mixed methods implementation research, Malaria Consortium assessed the feasibility and acceptability of co-implementing vitamin A supplementation with SMC in Dange-Shuni local government area, Sokoto state in 2019. Existing SMC implementation tools and job aids were revised and community health workers (CHWs) experienced in SMC delivery trained on determination of eligibility, administration of correct doses and identification of adverse drug reactions. SMC and vitamin A were delivered using the house-to-house approach for SMC. We estimated the coverage of Vitamin A and SMC; and assessed the potential impact of the integration on SMC implementation using data from questionnaires completed by trained interviewers from 188 and 197 randomly selected households at baseline and endline respectively. We assessed acceptability and feasibility through focus group discussions and key informant interviews; and carried out thematic analysis of transcripts. At endline, the proportion of children who received at least one dose of Vitamin A in the last six months increased significantly from 2% to 59% ($p<0.001$); there was no evidence of disruption to coverage or quality of SMC delivery with 70% of eligible children reached at baseline, increasing to 76% ($p=0.412$) at endline. Qualitative findings suggested feasibility of this co-implementation and general acceptability among CHWs, caregivers, health workers, programme managers and policy makers, with suggested areas of improvements in data and logistics management.

442

DETERMINING SEROPOSITIVITY - A REVIEW OF APPROACHES TO DEFINE SEROPREVALENCE WHEN USING MULTIPLEX BEAD ASSAYS TO ASSESS BURDEN OF TROPICAL DISEASES

YuYen Chan[1], Kimberly Fornace[1], Eric Rogier[2], Lindsey Wu[1], Ben F. Arnold[3], Jeffrey W. Priest[2], Diana L. Martin[2], Michelle Chang[2], Samuel E. Jean[4], Jackie Cook[1], Gillian Stresman[1], Chris Drakeley[1]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [3]*University of California, San Francisco, San Francisco, CA, United States,* [4]*Population Services International/Organisation Haïtienne de Marketing Social pour la Santé, Port-au-Prince, Haiti*

Serological surveys with multiplex bead assays can be used to assess seroprevalence to multiple pathogens simultaneously. However, there are several methods used to generate cut-off values for seropositivity that may lead to inconsistent interpretation of results. A literature review was conducted to describe the methods used to determine cut-off values

of data generated by multiplex bead assays. The implications of the choice of cut-off method on prevalence estimates were examined using data from Haiti and Malaysia as case studies. Articles were searched in PubMed and 291 relevant articles were identified that included the terms "serology", "cut-offs", and "multiplex bead assays". After exclusion criteria for relevancy to neglected tropical diseases or vaccine preventable diseases, 56 articles were examined. Several methods were used to determine seropositivity, including non-exposed and presumed unexposed populations, mixture models, receiver operating curves (ROC), quantiles, and visual inflection. When several of these cut-off methods were applied to the case study datasets, resulting prevalence estimates varied by method for several antigenic targets; lymphatic filariasis Bm14 and BM33 (non-exposed=5.65%, mixture model= 18.74%, quartile (75^{th}) =30.97%), *Strongyloides* NIE (non-exposed =2.95%, mixture model=8.99%, quartile (75^{th}) = 25.12%), and *Chlamydia trachomatis* Pgp3 (non-exposed =2.33%, mixture model= 42.95%, quartile (75^{th}) = 25.05%). Considerations for optimal cut-off approaches should include factors such as past precedents, transmission dynamics, and the immunological backgrounds of the population. In the absence of international standards for estimating seropositivity in a population, the use of consistent methods per disease allied with epidemiological data will improve comparability between settings and enable the assessment of changes over time.

443

CERVICAL CANCER: LATE PRESENTATION AND ASSOCIATED FACTORS AT MBARARA REGIONAL REFERRAL HOSPITAL

Sudi Mohamed

Mbarara University of Science and Technology, Mbarara, Uganda

Cervical cancer is the fourth most common cancer among women worldwide, ranking second in incidence and mortality in Lower Human Development Index countries. In East Africa, cervical cancer bears the highest burden of cancers in women globally. In Uganda, among the women with cervical cancer, 80% present with late-stage (FIGO Stage 2B to 4B). The stage at diagnosis of cervical cancer is reliant upon health-seeking by patients and appropriate actions by healthcare professionals. Late-stage of cervical cancer at diagnosis is correlated with lower survival rates. This study determined the proportion of women with late-stage cervical cancer at presentation and associated factors at Mbarara Regional Referral Hospital. We employed a cross-sectional study design and recruited 66 women with cervical cancer. The proportion of late-stage cervical cancer was calculated and association determined using Pearson Chi-square statistics, Fisher's exact test, bivariate analysis, and multivariate backward stepwise logistic regression approach. The proportion of late-stage cervical cancer (FIGO Stage 2B to 4B) was 68.2%. The mean age was 51.6 ±10.5 ranging between 29 and 68 years. Secondary education attainment (adjusted odds ratio 0.0, [0.00; 0.30], P=0.004) and awareness of cervical cancer screening (adjusted odds ratio=0.2, [0.03; 0.72], P=0.018) were protective for late-stage cervical cancer at presentation. Interval of ≥3 months from experiencing a symptom to seeking care was significantly associated with late-stage cervical cancer at presentation (adjusted odds ratio=16.6, [3.56; 77.07], P=0.000). Lack of awareness of cervical cancer screening, failure to attribute symptoms to cervical cancer, delays in seeking care contributed to late-stage cervical cancer at presentation. Future cervical cancer awareness and preventive interventions must be targeted to less-educated communities to reduce delays in seeking medical care for cervical cancer hence minimize morbidity and mortality and improve cancer survivorship.

444

ETHICAL CHALLENGES AND MORAL DISTRESS AMONG FIELD EPIDEMIOLOGISTS IN GLOBAL HEALTH

David G. Addiss[1], Emma Cooke[2], George Lopez[3], Angela Hilmers[4]

[1]*Task Force for Global Health, Atlanta, GA, United States,* [2]*Emory School of Medicine, Atlanta, GA, United States,* [3]*Emory University Rollins School of Public Health, Atlanta, GA, United States,* [4]*TEPHINET, Task Force for Global Health, Atlanta, GA, United States*

As frontline "disease detectives" and directors of public health programs, field epidemiologists play essential roles in protecting public health. Although ethical issues receive attention in medical settings and biomedical research, little is known about ethical challenges faced by epidemiologists in field settings, where the complexity of interests, influences, and pressures are considerably greater. Similarly, little is known about moral distress among field epidemiologists, defined as "a situation in the workplace where you know what the morally correct thing is to do, but circumstances or competing claims prevent you from doing it". In clinical settings, moral distress is strongly associated with empathy fatigue, burnout, reduced job retention, and disengagement. To address these knowledge gaps, in February 2019, members of TEPHIConnect, an online and mobile networking platform exclusively for Field Epidemiology Training Program (FETP) alumni, were invited to participate in an anonymous survey about ethical challenges and moral distress. Among 126 respondents from 54 countries, leading causes of ethical dilemmas involved inadequate informed consent (62%), inequitable allocation of resources (50%), and conflicts of interest (44%). These occur primarily in settings of disease outbreaks (60%); research (55%); and public health programs (46%). Work-related moral distress was reported by 91% of respondents, including 26% who experience it "frequently' or "almost always." Leading contributors to moral distress included excessive stress and work demands (30%) and lack of leadership support (25%). Field epidemiologists face a broad range of work-related ethical challenges, many of which are rooted within public health and political systems. Field epidemiologists also reported moral distress with alarming frequency. Little is known about how field epidemiologists cope with their moral distress. These findings suggest that a large unmet need exists among field epidemiologists for moral support and ethical training as well as for resources to address the unexpectedly high levels of moral distress reported.

445

CHARACTERIZATION OF THE ADAPTIVE IMMUNE RESPONSE ELICITED BY REPEATED EXPOSURE TO THE BITES OF AN INSECT VECTOR: IMPLICATIONS FOR VECTOR TRANSMITTED DISEASES

Chukwunonso O. Nzelu, Matheus B. Carneiro, Nathan C. Peters

Snyder Institute for Chronic Diseases, Departments of Microbiology, Immunology and Infectious Diseases, Cumming School of Medicine and Comparative Biology and Experimental Medicine, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada

Arthropod-transmitted diseases including malaria, Zika, Lyme's disease, and leishmaniasis are a significant public health problem in many regions of the world. During acquisition of a blood meal, hematophagous arthropods introduce an array of pharmacologically active salivary proteins and microbiota into the skin that influence hemostasis and inflammation. While it is known that these responses can influence the outcome of infection with an arthropod-transmitted pathogen, the immunological mechanisms underpinning this phenomenon and the immune response elicited by blood feeding are poorly understood. In the present study we investigated the immune response elicited by exposure to the bites of *Lutzomyia longipalpis* sand flies, an insect vector for the parasitic disease Leishmaniasis. As previously shown, short-term exposure to insect blood feeding activated salivary antigen-specific interferon (IFN)-gamma producing dermal-derived $CD4^{+}$Th1 cells. However, upon repeated exposure, the immune response underwent diversification at the population level to include multiple salivary antigen-

specific CD4+subsets (Th1, Th2, Th17 and T_{REG}), at both the dermal site of exposure and systemically. Analysis of the development of delayed type hypersensitivity (DTH) at the bite site during ongoing chronic exposure revealed four phases of bite-induced DTH, the last of which correlated with a high-degree of immunoregulation. Chronic exposure was associated with enhanced eosinophil recruitment to the skin, an alteration in the maturation of inflammatory monocytes towards an alternatively activated macrophage phenotype, and enhanced disease upon subsequent challenge with *Leishmania* plus salivary gland homogenates. These observations demonstrate how exposure to arthropod blood feeding can alter the dermal environment and how the 'host-vector-pathogen' relationship may impact the success of prophylactic and therapeutic intervention strategies against arthropod-transmitted diseases.

446

DECIPHERING OF MOLECULAR INTERACTIONS BETWEEN THE TRIPARTITE "*SIMULIUM DAMNOSUM* VECTOR, ENDOSYMBIOTIC BACTERIA AND *ONCHOCERCA VOLVULUS*": EXPLORATION OF THE POTENTIAL OF BACTERIAL SPECIES AS BIOLOGICAL TOOLS FOR THE DEVELOPMENT OF A NOVEL VECTOR CONTROL STRATEGY TO FIGHT ONCHOCERCIASIS IN AFRICA

Arnauld Efon Ekangouo

Centre for Research on Filariasis and other Tropical Diseases, Yaounde, Cameroon

Onchocerciasis remains endemic in sub-Saharan countries despite several decades of ivermectin-based mass chemotherapy. Factors related to this situation include very high transmission levels due to competent vectors. The vector control strategy was considered using larvicides, but was considerably limited by implementation difficulties, ecological pollution and the emergence of resistance within vector populations. Therefore, it remains necessary to develop more efficient and environmentally safe alternative strategies. This project aims to explore the potential of vector native bacterial species to be used as biological tools for the development of a novel fight strategy to ultimately interrupt the onchocerciasis transmission by producing biologically modified blackflies unable to transmit the parasite infection. Entomological survey was conducted in three onchocerciasis persisting foci in Cameroon. Transmission levels was assessed by measuring infectivity and infection rates through the *O. volvulus* detection on captured blackflies using quantitative PCR. Bacterial communities were screened from flies gut samples through high throughput sequencing of bacteria 16S rDNA and bioinformatics analysis allowed the taxonomic classification of bacterial species. The genetic diversity of blackflies and parasites was done through the characterisation of microsatellites makers. From 1,496 collected blackflies, Infestation rate was 10%, indicative of ongoing onchocerciasis transmission. Sequencing process revealed that gut bacterial communities was made up of 23 phyla and 210 genera and *Wolbachia* was the predominant genus (71%). This result is significantly larger than those of other arthropods of medical importance. *Serratia sp* and *Acidomonas* genera were significantly abundant among infected blackflies, whereas other genera as *Brevibacterium* were associated with the absence of infection. This study reveals that certain bacteria prevent the infestation of blackfly by *O. volvulus* and thus presents an interesting potential as a biological tool for the development of a new approach to fight against onchocerciasis.

448

DETECTION TIME LIMIT OF BLOOD MEAL HOST DEOXYRIBONUCLEIC ACID IN A TICK, RHIPICEPHALUS (BOOPHILUS) POST FEEDING.

Tanatswa X. Gara[1], Sungai Mazando[2]

[1]*Africa University, Mutare, Zimbabwe,* [2]*University of Zimbabwe, Harare, Zimbabwe*

The tick genus, *Rhipicephalus (Boophilus),* is a common haematophagous parasite that can be found feeding on mammals. This study aimed to examine the extent to which host DNA in a *Rhipicephalus* blood meal can be identified post feeding using a *cytochrome b* polymerase chain reaction (PCR) assay. Female adult ticks between 0.2 and 0.25 grams were collected from cows and were divided into 2 groups. One group was kept alive so as to analyse DNA degradation due to digestion and the other group was euthanized to analyse degradation due to the ticks' decomposition. Morphological changes and the presence/absence of cow DNA was analysed over time. Live ticks were analysed daily until they died and the dead ticks were analysed up to 290 days post feeding (PF). The ticks' gut contents were placed on Whatman® grade 903 filter paper DBS, were dried and DNA extraction was done using Chelex®. Amplification of a 561 base pair region on the *cytochrome b* gene of the cow mitochondrial DNA was achieved by PCR. The PCR products were visualized using agarose gel electrophoresis. Cow DNA was detected in the live ticks up to day 23 PF. The probability of successfully detecting cow DNA in the live ticks went down to at least 33% after day 15 PF. The live ticks started dying from day 40 PF and the last one died 52 days PF. Cow DNA was detected with bright bands in the dead ticks from day 1 PF to day 290 PF which is when the last sample was run, so the detection limit of DNA in dead ticks could not be determined. The persistence of host DNA in live ticks for up to 23 days PF and in dead ticks for more than 290 days PF shows the utility of ticks in forensic investigations. Methods used in this study can be used in forensic acarology to identify the host species of an arthropod when it is found at a crime scene.

450

SURVEILLANCE OF PLAGUE INFECTION IN MAMMALS AND FLEAS, MADAGASCAR, 2019

Soanandrasana Rahelinirina[1], Mireille Harimalala[2], Jerry Rakotoniaina[3], Romain Girod[2], Minoarisoa Rajerison[1]

[1]*Plague Unit, Institut Pasteur de Madagascar, Antananarivo, Madagascar,* [2]*Medical Entomology Unit, Institut Pasteur de Madagascar, Antananarivo, Madagascar,* [3]*Central Laboratory for Plague, Ministry of Public Health, Antananarivo, Madagascar*

Yersinia pestis, causative agent of plague, is usually transmitted to humans via bites from rodent fleas. In Madagascar, most surveillance and control efforts are based on human health. To estimate human risk and refine proactive plague prevention strategies, we conducted surveillance to describe exposure and infection in mammals and fleas. During December 2018 - May 2019, we captured small mammals (SM) using live traps in 5 districts (15 sites) in Madagascar ranging in plague endemicity from none to active foci. Sera collected from 16 dogs in the same sites were tested by ELISA for IgG anti-F1 antibodies. Fleas were removed from the SM, counted and identified to determine the average number of fleas per host (FI) and to detect *Y. pestis* DNA using PCR. We also collected SM blood and spleen specimens to detect IgG anti-F1 *Y. pestis* antibodies by ELISA and for testing *Y. pestis* F1 antigen using rapid diagnostic test (RDT). In addition, we collected free-living fleas in 430 households using candle traps. Reported rodent die-offs that characterize plague epizootics were noted. Of 557 SM captured and dissected (3 rodent and 1 shrew species), 3/166 (1.8%) spleens from an active focus, and 3/126 (2.4%) from a focus with no reports of human plague since 2015, were positive by RDT. Sera from 18/166 (10.8%) SM in an active focus, were IgG anti-F1 positive. Of 16 dogs, one, from a former plague focus, was IgG anti-F1 positive. A total of 1,547 SM fleas revealed *Xenopsylla cheopis* was the predominant rat flea. The FI ranged from 0.8 to 6.9 per SM, the highest of which was from a plague-free area. A total of 1,833 fleas were collected from households; *Pulex irritans* was the predominant species. None of the 1,320 rat, shrew and household fleas tested by PCR was *Y. pestis* positive. No rodent die-offs were reported. *Yersinia pestis* circulates at low rates in rodent populations in active, inactive and former plague foci. Our findings reinforce existing recommendations to adopt plague control measures including rodent, flea and dog surveillance even in inactive or former plague foci to avoid exposure to infected rodents and their fleas < for < and > for>.

451

TEMPERATURE MEDIATED EFFECTS ON VESICULAR STOMATITIS VIRUS INFECTION IN *CULICOIDES SONORENSIS* MIDGES

Paula Rozo-Lopez[1], Berlin Londono[1], Barbara Drolet[2]

[1]*Kansas State University, Manhattan, KS, United States,* [2]*United States Department of Agriculture, Manhattan, KS, United States*

Culicoides midges are well-known vectors of several viruses causing disease zoonotic diseases. Specifically, *Culicoides sonorensis* midges play a primary role in vesicular stomatitis virus (VSV) transmission. Environmental temperature impacts insect physiology and mediates the metabolic rate in midges causing alterations in oviposition, survival, and arboviral replication. In the midge, the virus is up taken during blood feeding and requires proliferation in multiple tissues of the vector before reaching transmission-related organs. In order for optimal transmission to occur during subsequent blood meals, virus replication and dissemination time should match the feeding-oviposition-feeding cycle. We investigated the effects of environmental temperatures on *C. sonorensis* survival, oviposition, and VSV titers in female midges held at constant temperatures of 20, 25, or 30°C. We found that midges held at 25°C showed the highest virus titers accompanied by a small reduction in survival and oviposition. Moreover, midges held at 20°C showed the highest rate of survival with a delay in oviposition and significantly lower virus titers. Midges held at 30°C showed the lowest survival rates and the fastest oviposition with only a mild increase in virus titers. Our results confirm that environmental temperature has direct implications on VSV epidemiology and it is relevant to study in relation to changing global climatic conditions.

452

EVALUATION OF A RAPID, POINT-OF-CARE MULTIPLEX IMMUNOCHROMATOGRAPHIC ASSAY FOR THE DIAGNOSIS OF ENTERIC FEVER

Shailendra Kumar[1], Ariana Nodoushani[2], Farhana Khanam[3], Alyssa T. DeCruz[1], Paul Lambotte[1], Robert Scott[1], Isaac I. Bogoch[4], Krista Vaidya[5], Stephen B. Calderwood[2], Taufiq R. Bhuiyan[3], Javan Esfandiari[1], Edward T. Ryan[2], Firdausi Qadri[3], Jason Andrews[6], **Richelle C. Charles**[2]

[1]*Chembio Diagnostic Systems, Inc, Medford, NY, United States,* [2]*Massachusetts General Hospital, Boston, MA, United States,* [3]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh,* [4]*University of Toronto, Toronto, ON, Canada,* [5]*Dhulikhel Hospital, Dhulikhel, Nepal,* [6]*Stanford University School of Medicine, Stanford, CA, United States*

There is a critical need for an improved rapid diagnostic for enteric fever. We have previously demonstrated that serum IgA responses targeting *Salmonella enterica* serovar Typhi hemolysin E (HlyE) and lipopolysaccharide (LPS) are able to discriminate patients with acute typhoid from healthy endemic area controls and from patients with other bacterial infections. We now have data demonstrating that IgA antibody responses against these antigens also work well for identifying patients with acute *S.* Paratyphi A infection. To develop a test for acute enteric fever detection, we have adapted a point-of-care immuno-chromatographic dual-path platform technology (DPP®), which improves on the traditional lateral flow technology by using separate sample and conjugate paths and a compact, portable reader, resulting in diagnostics with higher sensitivity and multiplexing abilities. In this analysis, we have compared our standard ELISA method to the DPP® method in detecting acute phase plasma/serum anti-HlyE and anti-LPS IgA antibodies in a cohort of patients with culture-confirmed *S.* Typhi (n=30) and Paratyphi A infection (n=20), healthy endemic controls (n=25), and febrile endemic controls (n=25). We found the DPP® measurements highly correlated with ELISA and both antigens had an AUC of 0.98 (sensitivity 92, specificity 94%) with all controls and an AUC of 0.98 (sensitivity of 90%, specificity 96%) with febrile endemic controls. Our results suggest that the point-of-care DPP® Typhoid System has high diagnostic accuracy for the rapid detection of enteric fever and warrants further evaluation.

454

COMPARISON OF STRATEGIES FOR TYPHOID CONJUGATE VACCINE INTRODUCTION IN INDIA: A GEOSPATIAL COST-EFFECTIVENESS MODELING STUDY

Theresa Ryckman[1], Arun S. Karthikeyan[2], Dilesh Kumar[2], Gagandeep Kang[2], Jeremy D. Goldhaber-Fiebert[1], Jacob John[2], Nathan C. Lo[3], Jason Andrews[1]

[1]*Stanford University School of Medicine, Stanford, CA, United States,* [2]*Christian Medical College Vellore, Vellore, India,* [3]*University of California, San Francisco, San Francisco, CA, United States*

Typhoid fever causes substantial global mortality, with almost half occurring in India. The new typhoid conjugate vaccine (TCV) has demonstrated safety and effectiveness and is recommended by the WHO for use in countries with high typhoid incidence. To inform an upcoming policy decision in India, there is a need for policy modeling to determine whether TCV introduction will be cost-effective and which strategies represent best value for money. We estimate the projected costs, health impact, and cost-effectiveness of TCV strategies in India using geospatial incidence estimates and a dynamic transmission compartmental model. We model both routine and one-time campaign vaccination strategies that target different age groups (1-year-olds, 1-15 year-olds, school-age children) and settings (entire state or urban areas only). The model is parameterized by disease and costing data from the Surveillance of Enteric Fever in India project, a recent multisite cohort and hybrid surveillance study across India. The primary outcome is cost-effectiveness, measured by incremental cost-effectiveness ratios (ICERs) benchmarked against India's 2018 Gross National Income per capita ($2020). Secondary outcomes include costs (in 2018 USD), disability adjusted life-years (DALYs), incidence, cases, and deaths. Preliminary results suggest that both routine and campaign vaccination strategies could be cost-effective in India, with ICERs in the range of $100 to $1000 per DALY averted for routine vaccination and $200 to $1500 per DALY averted for campaign vaccination, depending on the state and exact strategy. Vaccination limited to urban areas, which tend to have higher incidence, improved cost-effectiveness, with ICERs for routine and campaign strategies often falling below $100 and $200 per DALY averted, respectively. Over the first ten years of implementation, nationwide vaccination could avert between 11 and 19 million typhoid cases and tens of thousands of typhoid deaths, at a cost of $130-310 million annually, while vaccinating all urban areas would avert fewer cases (8-14 million) at lower cost ($10-70 million annually).

455

MACHINE LEARNING IDENTIFIES KEY RISK FACTORS OF LINEAR GROWTH FALTERING IN YOUNG CHILDREN WITH AND WITHOUT DIARRHEA

Sharia M. Ahmed[1], Benjamin Brintz[1], Patricia B. Pavlinac[2], James A. Platts-Mills[3], Karen L. Kotloff[4], Daniel T. Leung[1]

[1]*University of Utah, Salt Lake City, UT, United States,* [2]*University of Washington, Seattle, WA, United States,* [3]*University of Virginia, Charlottesville, VA, United States,* [4]*University of Maryland, Baltimore, MD, United States*

Stunting affected 144 million children less than 5 years old in 2019 and contributed to 15% of all deaths. Identification of those at highest risk will enable new interventions and targeted approaches. We used clinical and demographic data from the Global Enteric Multicenter Study (GEMS) study to build predictive models of linear growth faltering (decrease of ≥0.5 or ≥1.0 in height-for-age z-score [HAZ] at 60 day follow-up) in children ≤59 months presenting with moderate-to-severe diarrhea (MSD), and community controls, in Africa and Asia. We screened variables using random forests and assessed predictive performance with random forest regression and logistic regression using 5-fold cross-validation.

We stratified by occurrence of MSD and by age categories. Of the 7332 cases and 11963 controls, average HAZ at enrollment was -1.3, and 1650 (22.5%) of cases and 1909 (16.0%) of controls experienced growth faltering (≥0.5 ΔHAZ), and 345 (4.7%) of cases and 384 (3.2%) of controls had ≥1.0 ΔHAZ. Baseline HAZ, wealth, mid-upper arm circumference, respiratory rate, temperature, and age were predictive of growth faltering, regardless of age group, diarrhea status, and ΔHAZ cutoff. Other top predictors in both cases and controls included number of people in the house and number of sleeping rooms. Unique predictors in MSD cases <12mo and controls were duration of diarrhea and height at enrollment, respectively. AUCs up to 0.77 were observed for ≥1.0 ΔHAZ, but predictive ability was lower (AUC 0.64) for ≥0.5 ΔHAZ. While growth faltering was higher in MSD patients, most risk factors were consistent among MSD sufferers and healthy community controls, regardless of the severity of growth faltering. Our model accurately identified children at high risk of growth faltering using clinical features, living conditions, and age. To better reveal probabilistic relationships between growth faltering and risk factors, analysis using Bayesian networks are pending, and models based on conditional dependence of variables will be reported at time of abstract presentation.

456

IMPACTS OF *GIARDIA* CARRIAGE AND ENTERIC PATHOGEN CODETECTION ON CHILDREN IN THE VACCINE IMPACT ON DIARRHEA IN AFRICA STUDY: KENYA, THE GAMBIA, AND MALI, 2015-2018

Perrine Marcenac[1], Yiman Li[1], Sunkyung Kim[1], David M. Berendes[1], Graeme Prentice-Mott[1], Kristen Fagerli[1], Helen Powell[2], Irene N. Kasumba[2], Sharon M. Tennant[2], M. Jahangir Hossain[3], Syed M. Zaman[3], Henry Badji[3], Sarwar Golam[3], Richard Omore[4], John B. Ochieng[4], Jane Juma[4], Jennifer R. Verani[1], Alex Ondeng[4], Billy Ogwel[4], Marc-Alain Widdowson[1], Samba Sow[2], Sanogo Doh[5], Adama Mamby Keita[5], Awa Traore[5], Uma U. Onwuchekwa[5], Jie Liu[6], James A. Platts-Mills[6], Eric R. Houpt[6], Eric D. Mintz[1], Ciara O'Reilly[1], Karen L. Kotloff[2]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*University of Maryland, Baltimore, MD, United States,* [3]*MRC Unit The Gambia at the London School of Hygiene & Tropical Medicine, Banjul, Gambia,* [4]*Kenya Medical Research Institute, Kisumu, Kenya,* [5]*Center for Vaccine Development Mali, Bamako, Mali,* [6]*University of Virginia, Charlottesville, VA, United States*

In low- and middle-income settings, *Giardia* spp. is a prevalent pathogen with poorly understood associations with diarrhea and stunting. From 2015–2018, the Vaccine Impact on Diarrhea in Africa (VIDA) case-control study collected data to evaluate the etiology and clinical consequences of moderate-to-severe diarrhea (MSD) among children <5 years old in Kenya, The Gambia, and Mali. We assessed *Giardia* carriage among participants and examined codetection of other enteric pathogens via analysis of Taqman Array Card assays on collected stool. We evaluated associations between *Giardia* and stunting using a multivariable logistic regression considering each enteric pathogen codetection as a potential effect modifier and adjusting for age, gender, study site, caregiver's education, breastfeeding, and household assets. Possible correlations in groups within sites were considered using the Generalized Estimating Equation approach. Of 7,278 children tested for *Giardia*, 3,412 (47%) were positive—1,658 cases (49%), 1,754 controls (51%)—among whom 3,352 (98%) also tested positive for other enteric pathogens. The most common codetected pathogens were enteroaggregative *E. coli* (58%) and *Campylobacter* spp. (52%). Among cases of MSD, children with *Giardia* had higher odds of stunting (adjusted odds ratio (aOR): 1.20, 95% confidence interval (CI): 1.03–1.39). When examining whether interactions with specific enteric pathogens could alter this association, we found that the odds of stunting among cases with *Giardia* were not different in cases also carrying *Campylobacter coli/jejuni* (aOR: 0.91; 95% CI: 0.71–1.17). Among controls, children with *Giardia* had higher odds of stunting (aOR: 1.30, 95% CI: 1.11–1.52), and no interactions with other enteric pathogens altered this association. *Giardia* carriage was prevalent in children <5 years old within VIDA, and *Giardia* was commonly detected with other enteric pathogens. Results of these analyses support previous findings showing that *Giardia* carriage may be associated with stunting. Codetection of other enteric pathogens may alter these associations, particularly among children with MSD.

457

USE OF SOAP AND SAFE DISPOSAL OF CHILD'S FECES REDUCE TRANSMISSION AND CHILDREN'S EXPOSURE TO *CAMPYLOBACTER JEJUNI* IN THE KOLKATA, INDIA SITE OF THE GLOBAL ENTERIC MULTICENTER STUDY

Kurt Z. Long[1], Inong Gunanti[2], Byomesh Manna[3], Thandavarayan Ramamurthy[3], Suman Kanungo[4], Johanna Sanchez[2], James Nataro[5], Dilruba Nasrin[6], Myron Levine[6], Karn Kotloff[6]

[1]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [2]*Faculty of Medicine and Biomedical Sciences, University of Queensland,, Brisbane, Australia,* [3]*National Institute of Cholera and Enteric Diseases, Kolkata, India,* [4]*National Institute of Cholera and Enteric Diseases, Basel, India,* [5]*Department of Pediatrics, University of Virginia School of Medicine, Charlottesville, VA, United States,* [6]*Department of Medicine and Center for Vaccine Development, Baltimore, MD, United States*

Campylobacter prevalence has been rising exponentially across both developed and developing countries particularly, in countries like India, where *Campylobacter* spp. is reported as the second most common cause of bacterial enteritis. Identification of environmental reservoirs and transmission pathways for *Campylobacter* in South Asian communities can aid in the development of more effective intervention strategies to reduce this burden. Bayesian path analyses were used to model the effect of household pathogen transmission pathways on the risk of *C. jejuni* infections among children enrolled in the Kolkata, Indian component of the Global Enteric Multicenter Study (GEMS). Models were developed to test whether such potential pathogen reservoirs as water source and storage, sanitation facilities and animals in the compound were associated directly with greater *C. jejuni* infections or had indirect effects mediated by hygiene behaviors. There were 401 isolates of *C. jejuni* identified in stools collected from the 3,752 children enrolled in Kolkata. Treatment of water was significantly associated with a lower risk of *C. jejuni* infections while sharing bathroom facilities was associated with a greater risk. Storage of water was positively associated with *C. jejuni* infection if child's feces were not disposed of adequately but this risk was reduced if children's feces were disposed in a latrine or toilet. A greater number of children under the age of five in the household was associated with a greater risk of infections. This risk associated with number of children was reduced if households reported using soap when handwashing. An indirect inverse association of caretaker education with infection was found among households with a greater number of children when soap was used with handwashing and in households that stored water when child's feces were safely disposed. These results suggest that inadequate sanitation facilities, poor water management and social crowding can lead to greater transmission of *C. Jejuni* directly but that safe child feces disposal and use of soap can block such transmission.

458

EXCLUSIVE/PREDOMINANT BREASTFEEDING IS ASSOCIATED WITH LOWER RISK OF ENTEROPATHOGEN DETECTION: RESULTS FROM THE MAL-ED COHORT STUDY

Stephanie A. Richard[1], Benjamin J. McCormick[1], Laura E. Murray-Kolb[2], Estomih Mduma[3], Margaret Kosek[4], Eric Houpt[4], Pascal Bessong[5], Bruna L. Maciel[6], Aldo Lima[6], Ram Krishna Chandyo[7], Anuradha Bose[8], Tahmeed Ahmed[9], Laura E. Caulfield[10]

[1]*Fogarty International Center, NIH, Bethesda, MD, United States,* [2]*The Pennsylvania State University, University Park, PA, United States,* [3]*Haydom Lutheran Hospital, Haydom, United Republic of Tanzania,* [4]*University of Virginia, Charlottesville, VA, United States,* [5]*University of Venda, Thohoyandou, South Africa,* [6]*Federal University of Rio Grande do Norte,*

Fortaleza, Brazil, [7]Tribhuvan University, Kathmandu, Nepal, [8]Christian Medical College, Vellore, India, [9]icddr,b, Dhaka, Bangladesh, [10]The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Exclusive breastfeeding is recommended during the first 6 months of life for optimal nutrient intake and decreased exposure to food- and water-borne pathogens; however, in many low-income countries, other items are introduced to the diet before children reach 6 months of age. The MAL-ED study enrolled 1,868 newborns in eight sites, of whom 1,552 had breastfeeding and pathogen data through 6 months. Here we describe associations between breastfeeding status (exclusive or predominant (+ clear liquids)) and the presence of enteropathogens in participants' stool samples in the first 6 months of life. The median days of exclusive/predominant breastfeeding ranged from 11/22 days at the Naushahro Feroze, Pakistan site to 104/158 days at the Dhaka, Bangladesh site. Stool samples were collected each month, as well as during diarrheal episodes, and pathogens were isolated from both the monthly and the diarrheal stools. The most frequently identified pathogens were enteroaggregative *Escherichia coli* (EAEC), *Campylobacter spp.*, and *Giardia* (86%, 48%, and 33% of infants in the study had these respective pathogens at least once in the first 6 months of life). Based on logistic regression model results, exclusive/predominant breastfeeding in the seven days prior to stool sample collection was protective against detection of EAEC (OR 0.29, 95%CI 0.22 to 0.42), *Campylobacter* (OR 0.48, 95%CI 0.32 to 0.73), *Cryptosporidium* (OR 0.44, 95%CI 0.19 to 0.88) and norovirus (OR 0.44, 95%CI 0.20 to 0.49), but did not affect detection of other pathogens tested (rotavirus and *Giardia*). These models included an interaction with age, as well as sex and socioeconomic status and a random effect for study site and subject. These results demonstrate a protective effect of exclusive/predominant breastfeeding that is discernible for the two most commonly detected bacterial pathogens in these populations in this age range. These two pathogens have also been identified as risk factors for poor growth; therefore, this work underscores the importance of exclusive/predominant breastfeeding in early infancy in low-income countries.

459

MODULATION OF HUMAN DENDRITIC CELL FUNCTION THROUGH MICROFILARIAE-DERIVED EXTRACELLULAR VESICLES

Alessandra Ricciardi, Dhalia Metenou, Gayatri Sanku, Roshanak Tolouei Semnani, Thomas B. Nutman

Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, MD, United States

Intact microfilariae (mf) have previously been shown to alter human dendritic cell (DC) function most notably by altering cytokine production and inhibiting antigen presentation to T cells. Moreover, we and others have also shown that communication between mf and human monocytes occur partially through mf-derived extracellular vesicles (EVs). To understand the ramifications of the EV/cellular interaction, we isolated EVs (exosome-like in size and appearance) from mf and exposed them to monocyte derived human DC. These EVs were readily internalized by human DC as shown by confocal microscopy and induced the production of IL-10 along with IL-8, CCL5, and TNF-α . Interestingly, following exposure to the mf-derived EVs, the DCs when activated with LPS and Interferon-γ failed to produce significant levels of IL-12, a prototypic marker of DC activation. In addition, the inflammatory cytokines, CCL5 and IL-1β, were also decreased post-activation in DC exposed to mf-EVs. From a physiologic perspective, mf-free supernatants collected as excretory/secretory products could recapitulate the activity of the purified EVs; depletion of EVs from the mf supernatant through ultracentrifugation prevented most of the observed immune modulatory effects seen in DC. Collectively, our data demonstrate that mf release EVs that are internalized by host cells and modulate their function.

460

ENVIRONMENTAL ALLERGEN SENSITIZATION PROMOTES MARKED DIVERSITY IN HELMINTH-DRIVEN MEMORY CD4$^+$EFFECTOR TH2 CELLS IN HUMANS

Pedro Gazzinelli-Guimaraes[1], Philip Swanson[2], Thomas Nutman[1]

[1]*NAID, NIH, Bethesda, MD, United States,* [2]*VRC, NIAID, NIH, Bethesda, MD, United States*

A common feature between allergic disorders and helminth infections is their association with a Th2 immune response. We have previously demonstrated that allergic sensitization coincident with filarial infection drives a hyperreactive parasite antigen-specific Th2-dominated T cell response. To further characterize the heterogeneity and function of the Th2 cells, we used a self-organizing map tool (FlowSOM) to profile CD4$^+$ Th2 subsets driven by helminth and/or allergens based on multiparameter flow cytometry analysis of PBMCs collected from 24 filarial-infected subjects [Fil$^+$} with or without coincident house dust mite (HDM) sensitization (Filaria[Fil]$^+$HDM$^+$, n=12) and (Fil$^+$HDM$^-$, n=12) and 24 subjects without filarial infection (Fil$^-$HDM$^+$, n=12) and (Fil$^-$HDM$^-$, n=12). When all the 48 subjects' unstimulated CD4 T cells were analyzed by a two-level clustering algorithm, two memory CD4 T cell subsets demonstrated markedly different frequencies among the groups. The filarial infected groups had a marked significant increase in the frequency of CD4$^+$CD45RA$^-$CCR7$^-$CCR6$^+$CCR4$^+$CRTH2$^-$ T cells (70.7% in the Fil$^+$ vs 29.3% in the Fil$^-$, p=0.0173), as well as, in the frequency of CD4$^+$CD45RA$^-$CCR7$^-$CCR6$^-$CCR4$^+$CRTH2$^+$ T cells (72.5% in the Fil$^+$ vs 27.5% in the Fil$^-$, p<0.0001). The analysis of antigen specificity demonstrated that both subsets failed to increase in frequency when exposed to filarial parasite (BMA) antigen *in vitro*. BMA antigen stimulation, however, induced a marked increase in the frequency of another subset of memory Th2 cells (CD4$^+$CD45RA$^-$CCR7$^-$CCR4$^-$CRTH2$^-$CD69$^+$CD154$^+$OX40$^+$CD127$^+$IL-13$^+$IL-5$^+$IL-10$^+$TNF-a$^+$) in the filarial-infected subjects. Those with concomitant HDM sensitization had a further increase (60.9% in the Fil$^+$HDM$^+$; 28.02% in the Fil$^+$HDM$^-$; 4.18% in the Fil$^-$HDM$^+$ and 6.84% in the Fil$^-$HDM$^-$). Taken together, our data suggest that filarial infection drives a distinct diversity signature of memory Th2 cells in humans and that concomitant allergic sensitization alters even further this diversity. These findings are likely important in understanding the pathogenesis and/or regulation of helminth-allergy reactivity.

461

FILARIAL COINFECTION IS ASSOCIATED WITH HIGHER BACTERIAL BURDENS AND ALTERED PLASMA CYTOKINE AND CHEMOKINE RESPONSES IN TUBERCULOUS LYMPHADENITIS

Gokul Raj Kathamuthu

NIH-ICER-NIRT, Chennai, India

Filarial infections are known to modulate cytokine responses in pulmonary tuberculosis by their propensity to induce Type 2 and regulatory cytokines. However, very little is known about the effect of filarial infections on extra-pulmonary forms of tuberculosis. Thus, we have examined the effect of filarial infections on the plasma levels of various families of (IL-1, IL-12, γC and regulatory) cytokines and chemokines (CC and CXC) in tuberculous lymphadenitis co-infection. We also measured lymph node culture grades in order to assess the burden of *Mycobacterium tuberculosis* in the two study groups [Fil+ (n=67) and Fil- (n=109)]. Our data reveal that bacterial burden was significantly higher in Fil+ compared to Fil- individuals. Plasma levels of IL-1 family (IL-1α, IL-β, IL-18) cytokines were significantly reduced with the exception of IL-33 in Fil+ compared to Fil- individuals. Similarly, plasma levels of IL-12 family cytokines IL-12 and IL-23 were significantly reduced, while IL-35 was significantly elevated in Fil+ compared to Fil- individuals. Filarial infection was also associated with diminished levels of IL-2, IL-9 and enhanced levels of IL-4, IL-10 and IL-1Ra. Similarly, the Fil+ individuals were linked to elevated levels of different CC (CCL-1, CCL-2, CCL-3 CCL-11) and CXC (CXCL-2, CXCL-8, CXCL-9, CXCL-11) chemokines. Therefore, we conclude that filarial infections exert

powerful bystander effects on tuberculous lymphadenitis, effects including modulation of protective cytokines and chemokines with a direct impact on bacterial burdens.

462

AN ADENOVIRUS-VECTOR EXPRESSING CATHEPSIN B PROTECTS FROM SCHISTOSOMIASIS INFECTION IN A PRE-CLINICAL MODEL

Dilhan J. Perera[1], Adam S. Hassan[1], Mehdy Elahi[2], Christine Gadoury[2], Risini Weeratna[3], Renald Gilbert[2], Momar Ndao[1]

[1]*Research Institute McGill University Health Center, Montreal, QC, Canada,* [2]*National Research Council of Canada, Montreal, QC, Canada,* [3]*National Research Council of Canada, Ottawa, ON, Canada*

Schistosomiasis is one of the most prevalent parasitic diseases worldwide. With >700 million people are at risk of infection, this parasite causes debilitating illnesses which can last over 30 years. This project tested the protective ability of *Schistosoma mansoni* cathepsin B (SmCB) when delivered by a recombinant Adenovirus (AdV) as a vector. Our vaccine consisted of AdV-SmCB delivered intramuscularly, followed by two boosts of recombinant antigen. Upon immunization with our vaccine, we saw robust humoral responses in antigen specific IgG1 (endpoint titer: 34 822) and IgG2c (endpoint titer: 45 948) compared to the recombinant SmCB alone (IgG1 and IgG2c endpoint titer: 6 400). Flow cytometry analysis of splenocytes from vaccinated mice saw an increase of CD4+ and CD8+ T cells expressing IFNγ and an increased number of CD4+ T cells expressing IL2 and TNFα compared to the PBS control and recombinant antigen alone. Splenocyte supernatants were also assessed for cytokine expression and splenocytes from vaccinated animals demonstrated increased expression of many cytokines including: IL2, IL4, IL5, IL12, IFNγ, TNFα, and GM-CSF. Three weeks after the final immunizing boost, mice were challenged with 150 cercaria. Parasite burden reductions of 83.1%, 84.4%, and 91.8% were observed in adult worm, hepatic, and intestinal egg burdens respectively, compared to the PBS control. As Schistosoma larva travel through the lung, we additionally sought to harness the capability of our adenovirus to stimulate a mucosal response. A pilot study was conducted and found that a single immunization of AdV-SmCB delivered intranasally, reduced parasite burden by 79% in adult worms and more modestly, 55% and 56% in hepatic and intestinal eggs. Our recombinant adenovirus vaccine delivers schisto protection superior to the WHO standard of 40% parasite burden reduction. Not only would an effective vaccine for schisto benefit populations in endemic regions aiding interruption of disease transmission but it would also benefit international travelers to tropical regions.

463

NOVEL STATISTICAL APPROACHES TO IDENTIFY RISK FACTORS FOR SOIL-TRANSMITTED HELMINTH INFECTION

Jessica Aw[1], Naomi Clarke[2], Helen Mayfield[1], Colleen Lau[1], Alice Richardson[1], Susana Vaz Nery[2]

[1]*Australian National University, Canberra, Australia,* [2]*University of New South Wales, Sydney, Australia*

The identification of risk factors for soil-transmitted helminth (STH) infection has mainly relied on logistic regression models. However, the underlying assumption of independence between variables is not always satisfied. Previously demonstrated risk factors including water, sanitation and hygiene (WASH) and socioeconomic status are intrinsically linked. Although many studies have investigated such associations, the same risk factors are not consistently identified. Other data modelling techniques such as recursive partitioning and Bayesian networks utilise alternative approaches that can handle correlated data. There are no published studies comparing these methods with logistic regression in the context of STH infection. Using baseline cross-sectional data from school-aged children in the (S)WASH-D for Worms study, we compared the risk factors identified from modelling the same data using mixed-effects logistic regression, recursive partitioning and Bayesian networks. Outcomes were infection with *Ascaris* spp. and *Necator americanus*. Using logistic regression, fewer risk factors were significant overall and some were omitted due to correlation. For *Ascaris* spp., recursive partitioning mostly identified WASH and demographic risk factors, including handwashing, shoe-wearing and having children <5 years old in the household. On the other hand, Bayesian networks identified environmental risk factors including soil characteristics, climate and land attributes. For *N. americanus,* logistic regression and Bayesian networks both identified cleaning self with water after toileting and socioeconomic status as risk factors, while recursive partitioning identified having toilets with water as a risk factor. Overall, recursive partitioning produced easily interpretable classification trees and Bayesian networks provided insight into relationships between variables. This study adds to the limited body of evidence exploring alternative data modelling approaches in identifying risk factors for STH infection. Our findings suggest these approaches can provide novel insights for more robust interpretation.

464

EFFECTS OF ANNUAL VERSUS SEMIANNUAL MASS DRUG ADMINISTRATION FOR LYMPHATIC FILARIASIS ON HOOKWORM INFECTION IN CÔTE D'IVOIRE

Agodio Loukouri[1], Abdoulaye Méité[2], Benjamin G. Koudou[3], Charles W. Goss[4], Daphne Lew[4], Gary J. Weil[4], Eliézer K. N'Goran[1], Peter U. Fischer[4]

[1]*University Félix Houphouët Boigny, Abidjan, Côte D'Ivoire,* [2]*National Control Program of Neglected Tropical Diseases through Preventive Chemotherapy, Abidjan, Côte D'Ivoire,* [3]*Swiss Centre for Scientific Research, Abidjan, Côte D'Ivoire,* [4]*Washington University School of Medicine, Saint-Louis, MO, United States*

Mass drug administration (MDA) programs to eliminate lymphatic filariasis (LF) in western Africa use ivermectin (IVM, 0.2 mg/kg) plus albendazole (ALB, 400 mg) that have the potential to impact soil-transmitted helminth (STH). Integration of MDA efforts for LF, onchocerciasis, and STH should reduce costs. The current study compared the impact of five semiannual rounds of MDA versus three annual rounds of MDA on STH. The study was carried out from 2014 to 2017 in two health districts of eastern Côte d'Ivoire that were assigned to annual and semiannual MDA. STH data were collected from duplicate Kato-Katz method, prior to MDA and annually thereafter. Mixed logistic regression and mixed linear models were used to analyse data from repeated cross-sectional surveys. The number of participants tested ranged from 531 to 1112 in the annual MDA area and from 630 to 992 in the semiannual MDA area. The mean self-reported MDA compliance was 65.2% and 64.3% in the annual and semiannual treatment areas, respectively, without difference between treatment areas ($p > 0.05$). Hookworm was the most prevalent STH species in both areas (23.9% versus 12.4%) and the prevalence of the other species was less than 1%. The crude prevalence of hookworm dropped significantly, from 23.9% to 5.5% ($p < 0.001$), and from 12.4% to 1.9% ($p < 0.001$), respectively, in the annual and semiannual treatment areas. Intensity of hookworm decreased significantly, only in the annual MDA area ($p < 0.05$). Baseline moderate and heavy infections (1% and 1.3%) were reduced to 0% and 0.4% in the annual and semiannual treatment areas, respectively. Only the year 1 and year 2 re-examinations showed a difference, respectively, in intensity and prevalence between treatments ($p < 0.05$). Annual and semiannual rounds of community-wide MDA had similar impacts on hookworm infection in eastern Côte d'Ivoire. This is an underappreciated benefit of the LF elimination programs. Annual MDA was sufficient for controlling hookworm infection in these areas. Unlike school-based preventive chemotherapy, MDA targets the entire population above 4 years of age. Surveys are needed to assess the duration of the effect of MDA on STH.

465

PATTERNS OF INDIVIDUAL NON-TREATMENT DURING MULTIPLE ROUNDS OF COMMUNITY-WIDE MASS DRUG ADMINISTRATION FOR CONTROL OF SOIL-TRANSMITTED HELMINTHS IN THE TUMIKIA TRIAL, KENYA

William E. Oswald[1], Stella Kepha[1], Katherine E. Halliday[1], Carlos Mcharo[2], Th'uva Safari[2], Stefan Witek-McManus[1], Elizabeth Allen[1], Simon J. Brooker[1], Sammy M. Njenga[2], Charles S. Mwandawiro[2], Roy M. Anderson[3], Rachel L. Pullan[1]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*Eastern and Southern Africa Centre of International Parasite Control, Kenya Medical Research Institute, Nairobi, Kenya,* [3]*London Centre for Neglected Tropical Disease Research, Faculty of Medicine, Department of Infectious Disease Epidemiology, School of Public Health, St Mary's Campus, Imperial College London, London, United Kingdom*

Success of mass drug administration (MDA) for control of neglected tropical diseases depends on treatment coverage and patterns across rounds. There has been limited opportunity to explore these features within programmes at scale. Here we use routinely-collected individual-level treatment records collated for the TUMIKIA trial, conducted in coastal Kenya from 2015-2017, to estimate the extent of -- and factors associated with -- systematic non-treatment in the targeted population. The trial baseline population (N=36327) was linked with treatment records from four rounds of biannual community-wide MDA for soil-transmitted helminthiasis. We fit logistic regression models to estimate the association of non-treatment with treatment status during the previous round, controlling for identified predictors of non- treatment, and used multinomial logistic regression to identify factors associated with partial or no treatment versus complete treatment. Children aged 2--14 years and individuals aged 15+ years were more likely to remain untreated if they were not treated during the previous MDA. For children, school attendance and rural residence were protective against receiving partial and no treatment. Women were more likely than men to receive partial and no treatment, unless periods of pregnancy and recent childbirth were excluded; then, women more likely received complete treatment. Adults aged 20--25 years were most likely to receive partial or no treatment. We identified eligible populations who received incomplete treatment across multiple MDA rounds and found that this non-treatment did not occur at random. This finding has important implications for MDA programme effectiveness and will be increasingly important as prevalence reaches low levels.

466

EVALUATION OF THE COVERAGE OF PREVENTIVE CHEMOTHERAPY AGAINST SOIL-TRANSMITTED HELMINTHIASIS IN RWANDA

Eugene Ruberanziza[1], Jean Bosco Mbonigaba[1], Ladislas Nshimiyimana[1], Aimable Mbituyumuremyi[1], Jamie Tallant[2]

[1]*Rwanda Biomedical Center/Ministry of Health, Kigali, Rwanda,* [2]*The END Fund, New York, NY, United States*

Current monitoring of preventive chemotherapy coverage for neglected tropicale diseases (NTDs) is usually based on coverages reported by health facilities implementing the treatment campaigns. However, reported coverages are prone to errors resulting from incorrect estimates of the target population, weak health information systems, intentional inflation of the number of those treated and underreporting. In Rwanda, PC is implemented for soil-transmitted helminthiasis (STH) and schistosomiasis. This study aimed to estimate the coverage of mebendazole/albendazole against STH among targeted children aged 1-15 years, and determine if coverage exceeds the World Health Organisation (WHO)'s effective coverage (at least 75%) and validate the reported coverages. We used a community-based cross-sectional sudy. Eight districts were randomly selected, from which 30 villages were also randomly picked using the probability proportionate to estimated size. Using a systematic random sampling method, 1,140 households were selected among those having at least one eligible child. Interviews were conducted for 2548 children. The survey results revealed an overall, coverage at 89.5% (95% CI : 88.6-91.0) whilst the reported overall coverage in the study districts was 95.8%. The number of children who received deworming tablets but did not swallow them was negligible (0.3%). 31.3% of children who did not receive drugs reported not having seen distributors, 26.4% were not informed on mass drug administration, 20.3% were absent during the campaign and 14.2% reported drugs ran out. In conclusion, the present results provide with good insights and may suggest an evidence of a successful PC program. However, according to WHO guideline, this evaluation does not validate the reported coverage as the overall reported one falls outside the 95% confidence interval of the survey coverage. For better PC implementation, more efforts must be made to ensure drug distributors are regularly present at treatment sites, drugs are well estimated and available at all sites, and data collection tools are available and correctly filled.

468

NEW FOCI FOR INTESTINAL SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTHS INFECTION AFTER FIVE CONSECUTIVE YEARS OF MDA IN TWO DISTRICTS IN SOUTHERN ETHIOPIA

Zerihun Zerdo[1], Jean-Pierre Van geertruyden[2], Bastiaens Hilde[2], Sibyl Anthierens[2], Fekadu Massebo[1], Roy M Anderson[3], Justine Marshall[4], Misgun Shewangizaw[1], Yilma Chisha[1]

[1]*Arba Minch University, Arba Minch, Ethiopia,* [2]*University of Antwerp, Antwerp, Belgium,* [3]*London Centre for Neglected Tropical Disease Research (LCNTDR), London, United Kingdom,* [4]*London Centre for Neglected Tropical Disease Research, London, United Kingdom*

World Health Organization targeted to eliminate morbidity due to Schistosomiasis (SCH) and Soil-transmitted helmenthiasis (STH) among school-aged children (SAC) by 2020. The major strategy implemented in Ethiopia is providing periodic mass drug administration (MDA) based on finding from 5 schools in each district. This study aimed to estimate the prevalence of STH and SCH infections, intensity of infections and associated factors among SAC in two districts (previously known to be not endemic for SCH) in Southern Ethiopia, October to November 2019. It is part of cluster randomized controlled trial with nested process evaluation. Stool sample collected from 2254 children from 32 schools was diagnosed using Kato-Katz technique. Univariable and multivariable logistic regressions were used to assess association of infections with factors. The prevalence of STH infection was 33.6% (95% confidence interval (CI):31.6- 35.6). Intensity of infection was light (93.8%, 99.2% & 92.5%), moderate (4.7%, 0.8% & 7.5%) and heavy (5%, 0% & 7.5%) for hookworm, whipworm and Roundworms respectively. STH infection was lower in Uba Debretsehay district with Adjusted Odds Ratio (AOR) of 0.3 [95%CI: 0.2 to 0.5]; semi-urban area [AOR= 0.4; 95%CI: 0.2-0.8]; household head occupation of civil servant [AOR= 0.2; 95%CI: 0.1-0.4] and merchant [AOR= 0.5; 95%CI: 0.2-0.9]. Except four SAC, all infections of SCH were in Dara Mallo with prevalence of 35.1% [95%CI: 31.8-38.5%]. Of these, 42.9%, 33.7% and 23.4% were light, moderate and heavy infections respectively. SCH infection is positively influenced by living in semi-urban or urban area [AOR= 3.5; 95%CI: 2.1-6.1], female household head [AOR= 2.3; 95%CI: 1.1-2.4], literate household head [AOR= 1.6; 95%CI: 1.1-2.4] and mother of the child [AOR= 1.5; 95%CI: 1.03-2.6], farmer SAC mothers [AOR= 2.0; 95%CI: 1.02-3.7] and household having pit latrine without slab [AOR= 01.8; 95%CI: 1.3-2.6]. STH and SCH continue to be the health problem of children requiring (MDA) to be continued. The wide variation in prevalence of SCH infection in Dara Mallo district warrants for sub-district level mapping program and implementation of MDA.

469

A TOOL TO INVESTIGATE PERSISTENT HIGH TRANSMISSION OF STH INFECTIONS IN LOW PREVALENCE SETTINGS- CWW EXPERIENCE FROM BANGLADESH

Rubina Imtiaz[1], Ashraful Kabir[2], Abdullah Kawsar[3], Md. Jahirul Karim[1]

[1]*Children Without Worms, Decatur, GA, United States,* [2]*Children Without Worms, Dhaka, Bangladesh,* [3]*Elimination of Lymphatic Filariasis & STH Control Program, Dhaka, Bangladesh*

Following the introduction of systematic, school-based preventive chemotherapy (PCT) with donated medicines in 2006, approximately 20% of endemic countries have significantly lowered their national STH prevalence and intensity (ref. WHO-ESPEN, 2018 presentation). However, in these post-treatments, lower prevalence settings, surveys show further disease aggregation and likely presence of small, geographically circumscribed areas of continuous high infection transmission (or "hot spots"). Children Without Worms (CWW), a US-based NGO focused on promoting quality data use for STH and other PCT diseases, started collaborating with the Bangladeshi National Elimination of Lymphatic Filariasis and STH (ELFSTH) program in 2016. Multiple sub-national surveys were conducted (2017-2020) to map the current burden of STH following 10+ years of consistent high coverage with PCT of school-age children. These community-based surveys (presented elsewhere) highlighted several "hot spots". CWW had earlier developed three complementary checklists as a tool for national NTD programs to systematically explore the possible causes of failure to achieve an impact on infection transmission following multiple treatment rounds. In 2019, CWW and the ELFSTH program staff applied these checklists to two selected "hot spots" in Bangladesh. This initial field application of the tool led to adaptations in the tool itself to include a qualitative component that assessed shortfalls at the local health service delivery and worker levels and assessed community barriers to PCT. The results will be helpful for the national program to strengthen the local response capacity, resources, and monitoring to support scaling up the intervention in these areas to the community-level (previously through the schools only). This tool and approach are helpful and applicable to other NTDs that rely on PCT as their key intervention, and to additional countries that have consistently achieved high PCT coverage and are now looking to map and mop up localized, high-infection-transmission areas.

470

ANTIBODY LEVELS IN *TRYPANOSOMA CRUZI* INFECTION CORRELATE WITH PARASITEMIA AND CARDIOMYOPATHY: DATA FROM THE REDS-II COHORT

Lewis Fletcher Buss, Ester C. Sabino

Faculdade de Medicina da Universidade de São Paulo, Sao Paulo, Brazil

We present associations between antibody titers, parasitemia and cardiac involvement in the REDS-II cohort of chronic Chagas disease patients. This analysis included 499 *T. cruzi*-seropositive blood donors and 101 patients with established Chagasic cardiomyopathy. Blood was taken for quantitative serology (based on crude parasite antigens, expressed as signal-to-cutoff), PCR and brain naturetic peptide (BNP). ECG and echocardiogram were interpreted blind to clinical status. We excluded 49 subjects with prior benznidazole treatment. The proportion of subjects with a positive PCR increased across the dynamic range of the serologic assay (S/CO < 3, 0% positive; 3-3.9, 14%; 4-4.9, 48%; 5-5.9, 56%; 6-6.9, 77%; ≥7, 71%; $p < 0.001$). Blood donors with cardiomyopathy had higher antibody titers (median 6.6; range 3.2-8.6) than those without (6.2; 0.3-8.6; p-value < 0.001) – in particular note the lower bound of the ranges. Simple linear regression showed a negative association between antibody level and left ventricular ejection fraction ($\beta = -0.57$, $p = 0.02$) and a positive association with left ventricular end-diastolic diameter ($\beta = 0.36$, $p = 0.002$). Similarly, there was a linear relationship between antibody titer and the log of BNP ($\beta = 0.05$, $p < 0.001$). In conclusion, the probability of PCR positivity is a continuous function of antibody level, both presumably reflecting tissue parasite burden. Antibody level is associated with cardiac disease, also supporting this hypothesis.

472

RAPID DIAGNOSTIC TESTS COMBINED WITH TREATMENT WITH A SINGLE-DOSE DRUG SPEEDS UP THE ELIMINATION OF GAMBIENSE HUMAN AFRICAN TRYPANOSOMIASIS

Ron Crump[1], Ching-I Huang[1], Erick Mwamba Miaka[2], Kat S. Rock[1]

[1]*The University of Warwick, Coventry, United Kingdom,* [2]*PNLTHA, Kinshasa, Democratic Republic of the Congo*

An oral drug, acoziborale, is undergoing clinical trials for the treatment of gambiense human African trypanosomiasis (gHAT) and could potentially be available soon. gHAT is approaching elimination, but the remaining cases are primarily located in hard-to-reach populations where health clinics are scarce. It is hoped that acoziborale will be a safe, single-dose cure for gHAT, in which case acoziborale could change the paradigm of gHAT control. This would be especially true for screening of symptomatic and asymptomatic individuals by mobile diagnostic teams visiting villages. It may be possible that the non-toxic compound used in acoziborale may allow mobile screening teams to treat individuals who test positive using rapid diagnostic tests (RDT) without parasitological confirmation and on an outpatient basis in their villages - a marked improvement over existing complicated courses of treatment. Used on their own, RDTs for gHAT have higher sensitivity and lower specificity than the current diagnostic algorithms (which include microscopy for confirmation), which would result in fewer false negative and more false positive diagnoses. In this study we adapt a previously developed mathematical model to predict the drug use and levels of overtreatment which may occur in strategies as outlined above. We focus on the Democratic Republic of Congo (DRC), which has around 84% of the current case burden and for which we have previously parameterised the model. Our results show that the availability of a drug which is safe to use in an RDT+treat active screening framework could speed up the time to elimination due to the sensitivity gains, however data collected will become harder to interpret, with the numbers of treated people greatly increasing. This work suggests that a verification test (such as trypanolysis) may be extremely valuable to monitor the progress towards elimination by retaining a perspective on the remaining true infections.

473

SPATIOTEMPORAL DISTRIBUTION OF VISCERAL LEISHMANIASIS WITH CONSIDERATION OF ENVIRONMENTAL RISK FACTORS, MINAS GERAIS, BRAZIL, 2012-2018

Shelby L. Lyons[1], Julie A. Clennon[1], José A. Ferreira[2], Jessica K. Fairley[3], Uriel Kitron[4]

[1]*Emory Rollins School of Public Health, Atlanta, GA, United States,* [2]*Faculdade da Saúde e Ecologia Humana, Vespasiano, Brazil,* [3]*Emory University School of Medicine, Atlanta, GA, United States,* [4]*Emory University, Atlanta, GA, United States*

Visceral leishmaniasis (VL) is endemic in Brazil, representing 97% of all VL cases in the Americas. Previous studies have described the distribution of visceral leishmaniasis cases in Minas Gerais (MG), Brazil, indicating spatial heterogeneity at a mesoregion level. Environmental factors such as urbanization, rainfall, vegetation, and temperature, have been found to be associated with VL, but data are limited for MG. This study describes the incidence of VL in MG, evaluates environmental factors impacting VL incidence in the state, and reviews areas of high risk and clustering of the disease. A spatiotemporal ecological study utilizing confirmed cases of VL by municipality through the Brazilian Notifiable Disease Information System (SINAN) was conducted from January 2012 through December 2018. Maps of VL cumulative and annual incidence were created to evaluate the spatial heterogeneity of disease across the state. Multivariate negative binomial regression models were developed for the overall time period, as well as annually to evaluate the association between

minimum temperature, maximum temperature, total precipitation, urban residence, and normalized vegetation, and VL incidence. Local indicator of spatial autocorrelation (LISA) and Kulldorff spatial scan statistics were conducted to evaluate clustering. Over the study period 3,501 cases of VL were reported across Minas Gerais, for a cumulative incidence of 16.5 cases per 100,000 persons. The average annual incidence rate in MG was 3.8 cases per 100,000 persons. Urban residence displayed consistent significant association with VL incidence in MG. Maximum temperature, total precipitation, and normalized vegetation indices were also found to be significantly associated with VL in the overall model for 2012-2018. Significant clustering of VL was observed in northern MG. This study offers insight into the distribution of VL in Minas Gerais, how environmental factors are impacting the disease in the state, and where clustering is most likely occurring. These results help offer guidance on prevention and control efforts for VL in Minas Gerais, Brazil.

476

ESTIMATING THE POTENTIAL IMPACT ON ELIMINATING TRANSMISSION OF SLEEPING SICKNESS DUE TO THE INTERRUPTION OF ACTIVITIES DURING THE COVID-19 PANDEMIC

Ching-I Huang[1], Maryam Aliee[1], Ronald Crump[1], Soledad Castaño[2], Chris Davis[1], Erick Mwamba Miaka[3], Matt Keeling[1], Nakul Chitnis[4], Kat Rock[1]

[1]*University of Warwick, Coventry, United Kingdom,* [2]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [3]*Programme National de Lutte contre la Trypanosomiase Humaine Africaine, Kinshasa, Democratic Republic of the Congo,* [4]*Swiss Tropical and Public Health Institute, Basel, Swaziland*

Gambiense human African trypanosomiasis (gHAT; sleeping sickness) is targeted for elimination of transmission (EOT) by 2030. In 2018 the Democratic Republic of the Congo (DRC) accounted for ~70% of global cases reported in 12 countries in West and Central Africa. Whilst the national control programme in the DRC (PNLTHA) has been striving towards the EOT goal, the first novel Coronavirus disease 2019 (COVID-19) cases were reported in the DRC on 10th March 2020. Although most of the initial COVID-19 cases were identified outside the gHAT foci, Kwilu province which has a high gHAT burden and is a focus of intervention efforts, is one of the first five provinces to report COVID-19 cases. International and internal travel bans quickly resulted in suspension of some gHAT interventions. Although gHAT is a slow disease, the concern of increases in transmission or resurgence due to the interruption of medical interventions during the COVID-19 pandemic leads us to this study. In this paper we use our previously fitted gHAT models to simulate the predicted impact of different possible interruption scenarios of gHAT activities due to the COVID-19 pandemic on the delay of EOT in 157 health zones in the DRC. Our results show that the impact of interruption of control activities for one or two years may delay EOT by up to three years on average. Despite the fact that achieving EOT by 2030 is less likely in high-risk health zones regardless of interruption, the interruption would translate into higher levels of disease induced mortality in the coming years. Interruption of passive surveillance in particular could be very detrimental to individuals living in at-risk areas and lead to largest EOT delays. We present various mitigation strategies including increased active screening and vector control to explore how the programme could get back on track after interruption.

477

ADAPTATION TO MOSQUITO VECTOR SPECIES IMPACTS EVOLUTION OF *PLASMODIUM FALCIPARUM*

Ankit Dwivedi[1], Alvaro Molina-Cruz[2], Kara A. Moser[1], Drissa Coulibaly[3], Mahamadou A. Thera[3], Chanthap Lon[4], Lek Dysoley[5], Stuart D. Tyner[4], David L. Saunders[6], Myaing M. Nyunt[7], Christopher V. Plowe[7], Miriam K. Laufer[8], Mark A. Travassos[8], Shannon Takala-Harrison[8], Carolina Barillas Mury[2], Joana C. Silva[1]

[1]*Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD, United States,* [2]*National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States,* [3]*Malaria Research and Training Center, University of Science, Techniques and Technologies, Bamako, Mali,* [4]*Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand,* [5]*The National Center for Parasitology, Entomology and Malaria Control, Ministry of Health, Phnom Penh, Cambodia,* [6]*US Army Research Institute of Infectious Diseases, Ft. Detrick, MD, United States,* [7]*Duke Global Health Institute, Duke University, Durham, NC, United States,* [8]*Malaria Research Program, Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States*

Plasmodium falciparum causes the most severe form of human malaria, and the most common worldwide. Over 60 geographically-localized *Anopheline* mosquito species transmit malaria to humans. *P. falciparum* evolved from *P. praefalciparum* after a gorilla-to-human host jump, and *P. reichenowi* is a close outgroup to both species. Recent observations by our groups, through both *P. falciparum* comparative genome analysis and the study of parasite-mosquito interactions, suggest that the vector may have an underappreciated impact on the evolution of the parasite. For example, a *Pfs47* haplotype present in Southeast Asia (SEA) and Papua New Guinea is similar to the *P. praefalciparum* haplotype and is absent in Africa. Also, a structural variant in AP2-G present in SEA and the outgroup species, is absent in Africa. We hypothesize that adaptation to the vector contributes to regional genetic differences between *P. falciparum* populations. We took advantage of the overlap in vectors that transmit *P. falciparum* sister taxa and *P. falciparum* vectors in Southeast Asia (SEA), to test if fixed differences are observed between African and SEA *P. falciparum*, in which the latter retains the ancestral state and are enriched for vector related functions. Analyses were based on whole genome sequence data for ~1100 global *P. falciparum* isolates/strains, 19 long read-based *P. falciparum* genome assemblies from Africa and SEA and *P. praefalciparum* and *P. reichnowi* whole genome samples. We observed that ~40% of highly differentiated SNPs (F_{ST}>0.9) are nearly fixed in SEA, with identical character state in the outgroups and different in Africa. These SNPs are present preferentially in genes with higher expression (≥ 2-fold change) in gametocytes or other mosquito developmental stages compared to vertebrate stages. Further investigation of functional processes and metabolic pathways associated with the loci is underway. Identification of processes critical for vector competence may unveil candidate loci for malaria vaccine development.

478

PERSISTENCE OF GENETICALLY IDENTICAL PARASITES ACROSS MULTIPLE TRANSMISSION SEASONS AND EVIDENCE OF CO-TRANSMISSION IN THIÈS, SÉNÉGAL BETWEEN 2006 AND 2019

Stephen F. Schaffner[1], Rachel F. Daniels[2], Yaye Die Ndiaye[3], Katherine Figueroa[1], Angela M. Early[1], Aida S. Badiane[4], Awa B. Deme[3], Fatou Ba Fall[5], Medoune Ndiop[5], Alioune Badara Gueye[6], Ibrahima Diallo[5], Moustapha Cisse[5], Claudia R. Taccheri[2], Albert Lee[7], Caitlin Bever[7], Joshua L. Proctor[7], Doudou Sene[5], Daniel L. Hartl[8], Bronwyn MacInnis[1], Sarah K. Volkman[2], Daouda Ndiaye[4], Dyann F. Wirth[2]

[1]*Broad Institute, Cambridge, MA, United States,* [2]*Harvard T.H. Chan School of Public Health, Boston, MA, United States,* [3]*Dantec Teaching and Research Hospital, Dakar, Senegal,* [4]*Cheikh Anta Diop University, Dakar,*

Senegal, [5]Senegal National Malaria Control Program, Dakar, Senegal, [6]Senegal National Malaria Control Programe, Dakar, Senegal, [7]Institute for Disease Modelling, Bellevue, WA, United States, [8]Harvard University, Cambridge, MA, United States

In an ongoing study in Thiès, Senegal, genetic analysis has detected highly related *Plasmodium falciparum* infections that have persisted across multiple transmission seasons. We hypothesized that these persistent parasite lineages could reveal patterns of genetic outcrossing or inbreeding that were informative about the transmission dynamics of this parasite population. In this study, analyzed 1,700 samples obtained from patients with uncomplicated malaria infections that were seen at the SLAP clinic in Thiès, Sénégal between 2006 and 2019; these samples were genotyped at 24 independent high minor-allele-frequency single-nucleotide positions. We also analyzed full genome sequence data from 150 of these samples, focusing on parasite infections that are likely to contain a single parasite genome (i.e., are monogenomic). Epidemiological modeling of genetic patterns revealed transmission declines and rebounds during this period, reflected in the fraction of highly related pairs within a transmission season, which varied 50-fold. Relatedness across successive years was on average lower by a factor of two compared to that within a season. Genotyping analysis revealed 60 distinct parasite lineages that persisted for more than one transmission season, with a maximum observed duration of 11 years. Comparison between genotyping and sequencing confirmed that barcode identity, characterized by a perfect match between pairwise comparisons, consistently identifies highly related parasites. We also confirmed that identical clones persist across multiple years of transmission despite some level of ongoing outcrossing in the population. Sequencing analysis detects both first- and second-degree relatives among the parasite population, as well as more inbred pairs that suggest the effects of ongoing co-transmission of infections in this population. Epidemiological modeling of population genetic parasite data can be used to reveal important patterns of transmission dynamics to better understand how parasite infections in a population are derived, shared, and maintained.

479

REDUCED *PLASMODIUM FALCIPARUM* DIVERSITY AND INCREASED GAMETOCYTE CARRIAGE AFTER A MALARIA ELIMINATION INITIATIVE IN SOUTHERN MOZAMBIQUE

Himanshu Gupta[1], Beatriz Galatas[1], Gloria Matambisso[2], Carlos Ruiz-Arenas[1], Lidia Nhamussua[2], Wilson Simone[2], Arlindo Chidimatembue[2], Pau Cisteró[1], Juan R. González[1], Regina Rabinovich[1], Pedro Alonso[1], Francisco Saute[2], Pedro Aide[2], **Alfredo Mayor**[1]

[1]*Barcelona Institute for Global Health (ISGlobal), Barcelona, Spain,* [2]*Manhiça Health Research Center (CISM), Manhiça, Mozambique*

Recent shifts in global health policy have renewed interests on the feasibility and impact of mass drug administration (MDA) programs against malaria. Such large-scale programs, which aim to quickly reduce the parasite biomass in a community and to prevent new infections for a certain period, can exert prolonged novel selection pressures on *Plasmodium falciparum* (*Pf*) parasites. In Magude District (Southern Mozambique), four MDA rounds with dihydroartemisinin-piperaquine and annual indoor residual spraying led to a decline of all-age *Pf* prevalence by rapid diagnostic test from 9.1% in May 2015 to 2.6% in May 2017. Here we compared parasite genetic diversity and relatedness (deep sequencing targeting *pfcsp* and *pfama1* amplicons) and the levels of gametocyte-specific markers (reverse transcription qPCR) *in Pf* isolates collected before and after the intervention. The relative transcript number of *pfs25* (female gametocyte marker), *ap2g* (master regulator of sexual differentiation) and *gexp02* (a marker for circulating sexual rings) was higher after (n=50) than before (n=57) the intervention ($p<0.05$). The multiplicity of infection (number of haplotypes detected in a sample), Shannon index (which simultaneously considers the number and proportions of haplotypes in a sample) and the carriage of monoclonal infections were also reduced. In contrast, genetic relatedness between infections, quantified as the presence of coincident major haplotypes, increased after the intervention. Overall, these results show that the intervention led to a reduction of within-host diversity and an increase in between-host relatedness. Such a reduction in diversity may increase the potential value of genomics to detect malaria imported cases in settings approaching elimination. Moreover, the intervention was associated with an increase of gametocyte carriage, suggesting that malaria parasites may adapt to large reductions in malaria transmission through a higher investment in transmission to new hosts. Further studies are required to assess if reductions of within-host competition between parasite clones may lead to changes in sexual investment.

480

SPATIAL PATTERNS OF FALCIPARUM MALARIA GENETIC RELATEDNESS DRIVEN BY HUMAN MOVEMENT IN THE DEMOCRATIC REPUBLIC OF THE CONGO

Nicholas F. Brazeau[1], William Weir[1], Amy Wesolowski[2], Oliver J. Watson[3], Andrew P. Morgan[4], Azra C. Ghani[5], Jonathan Juliano[1], Steven Meshnick[6], Robert Verity[5]

[1]*University of North Carolina School of Medicine, Chapel Hill, NC, United States,* [2]*John Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [3]*Brown University, Providence, RI, United States,* [4]*Duke University, Durham, NC, United States,* [5]*Medical Research Council Centre for Outbreak Analysis and Modelling, London, United Kingdom,* [6]*Gillings School of Global Public Health, Chapel Hill, NC, United States*

Identity by descent (IBD) analyses have reemerged as a rich approach for detecting patterns of isolation by distance and transmission intensity among malaria parasites. However, geographic space is frequently only considered as greater-circle distance, which likely approximates dispersion of parasites by mosquitos and not necessarily by hosts. Using 1,111 samples genotyped at nearly 1,800 loci from across the Democratic Republic of the Congo (DRC), we analyzed the decay of genetic and spatial relatedness across four measures of space: (1) greater-circle distance, (2) road distance, (3) river distance, and (4) air-travel "flight" distance. We found that road distance best explained genetic relatedness in the DRC under a classic isolation by distance model. This finding was further explored with a gradient-descent approach measuring genetic inbreeding across space and by correlations between the genetic relatedness and road network topologies (*i.e.* network centrality measures). In addition, investigation of highly related pairs revealed that pairs from different sampling locations were found at distances that exceed expected maximum anopheline mosquito flight distances. This suggests that the dispersion of falciparum parasites across the DRC is being driven by human movement instead of mosquito movement. Characterization of how *P. falciparum* parasites are migrating in the DRC and understanding patterns of gene-flow can direct policymakers where antimalarial interventions would be most effective.

481

THE IMPACT OF ANTIMALARIAL DRUG RESISTANCE ON ESTIMATION OF MALARIA PARASITE MIGRATION AND EFFECTIVE POPULATION SIZE

Bing Guo[1], Zalak Shah[1], Yao Li[2], Joana C. Silva[1], Zackary Park[3], Huy Rekol[4], Soklyda Chann[5], Michele D. Spring[5], Mariusz Wojnarski[5], David L. Saunders[6], Philip L. Smith[5], Chanthap Lon[5], Brian A. Vesely[5], Jessica T. Lin[3], Norman C. Waters[5], Kathleen E. Stewart[2], Timothy D. O'Connor[1], Shannon Takala-Harrison[1]

[1]*University of Maryland School of Medicine, Baltimore, MD, United States,* [2]*University of Maryland, College Park, MD, United States,* [3]*University of North Carolina at Chapel Hill, Chapel Hill, NC, United States,* [4]*National Center for Parasitology Entomology and Malaria Control, Phnom Penh, Cambodia,* [5]*Armed Forces Research Institute of Medicine Sciences, Bangkok, Thailand,* [6]*US Army Research Institute of Infectious Diseases, Ft. Detrick, MD, United States*

The emergence of multidrug-resistant *Plasmodium falciparum* in Southeast Asia has prompted intensive malaria elimination efforts. Estimates of

parasite population demography can guide allocation of resources and evaluation of progress toward elimination. However, metrics like identity-by-descent (IBD) used to infer parasite population demography are affected by selective sweeps resulting from drug resistance. We examined whole genome sequence data from 276 *P. falciparum* infections from northern Cambodia from 2013-2017 to understand the impact of selective sweeps on IBD-based inferences of parasite population demography. IBD segments (>2cM) between pairs of isolates were inferred using Beagle 4.1. The proportion of pairs with IBD sharing was estimated, and IBD peaks were defined as genomic regions with proportion >2 standard deviations above the chromosome mean. Inferred IBD segments were analyzed using IBDne to estimate effective population size (Ne) over the last 100 generations (1-7 generations/year). We observed a high proportion of sample pairs with IBD sharing (2.0% ± 0.6%), with IBD segments distributed genome-wide. IBD peaks did not include known drug resistance genes. Using all IBD segments, estimates of Ne decreased until 20-30 generations ago, followed by a plateau in the last 10 generations. After removing IBD segments associated with known drug resistance genes or IBD peaks, using both conservative and aggressive approaches, estimates of Ne increased up to 2-fold in the last 8-10 generations; however, the overall pattern of Ne over the past 100 generations remained similar. Our results indicate that drug resistance drives only part of the most recent change in estimated Ne of *P. falciparum* in this geographic area. In addition, the high genome-wide IBD observed in isolates from this area may present challenges for inference of population demography using IBD-based metrics. Ongoing efforts, including selective sweep simulation and migration surface analysis with both IBD-based and non-IBD-based tools, will provide further insights into the impact of selective sweeps on estimates of parasite population demography.

482

TEMPORAL AND SPATIAL ANALYSIS OF *PLASMODIUM FALCIPARUM* GENOMICS REVEALS PATTERNS OF CONNECTIVITY IN A LOW-TRANSMISSION SETTING IN SOUTHERN PROVINCE, ZAMBIA

Kara A. Moser[1], Ozkan Aydemir[2], Jeffrey A. Bailey[2], Chris M. Hennelly[1], Patrick W. Marsh[2], Amy Wesolowski[3], Tamaki Kobayashi[3], Tim Shields[3], Harry Hamapumbu[4], Michael Musonda[4], Ben Katowa[4], Japhet Matoba[4], Jennifer C. Stevenson[4], Douglas E. Norris[5], Philip E. Thuma[4], Steven R. Meshnick[6], William Moss[3], Jonathan J. Juliano[1]

[1]*Institute for Global Health and Infectious Diseases, University of North Carolina Chapel Hill, Chapel Hill, NC, United States,* [2]*Department of Pathology and Laboratory Medicine, Brown University, Providence, RI, United States,* [3]*Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [4]*Macha Research Trust, Choma District, Zambia,* [5]*Department of Molecular Microbiology and Immunology, Johns Hopkins Malaria Research Institute, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [6]*Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina Chapel Hill, Chapel Hill, NC, United States*

As malaria endemic regions transition from high to low transmission, understanding temporal and spatial dynamics of ongoing transmission will be critical to inform effective interventions and elimination strategies, particularly at operational-level spatial scales. Increasingly, parasite genomics are being used as a tool to monitor changes in epidemiologic trends. Southern Province, Zambia has had a dramatic decrease in cases over the past two decades, and is now a low-transmission setting with seasonal malaria. To investigate parasite population dynamics in this region, we genotyped 442 *Plasmodium falciparum* samples using molecular inversion probes at 1,800 positions across the genome, using dried blood spots collected from 2012-2018 as a part of passive case detection of symptomatic individuals presenting to 8 health centers in and around the catchment area of Macha Mission Hospital in Choma District, Southern Province. We identified signals of population size fluctuation over the course of individual transmission seasons (increases in complexity of infection; decreases in inbreeding coefficients), despite being an overall low-transmission region, suggesting a ramp-up of malaria transmission from a season's beginning. Highly-related parasite pairs (inbreeding coefficients > 0.9) were sustained not only over the course of individual seasons, but were also identified between seasons, suggesting parasites from one season are "seeding" the next. Despite the small spatial scale of the study (< 100 km), we identified an inverse relationship between genetic relatedness of parasite pairs and distance between health centers, as well as increased relatedness between specific health centers. Modeling approaches which integrated genomic and epidemiological data identified spatial and temporal hotspots of transmission which could be targeted by control programs. These results, leveraging both genomic and epidemiological data, provide a comprehensive picture of fluctuations in parasite populations in this pre-elimination setting of southern Zambia.

483

PLASMODIUM FALCIPARUM HISTIDINE-RICH PROTEIN 2 AND 3 GENE DELETIONS IN ETHIOPIA: CONFIRMATION AND MAPPING USING A NOVEL DEEP SEQUENCING APPROACH

SindewMekasha Feleke[1], Emily N. Reichert[2], Ozkan Aydemir[3], Hussein Mohammed[1], Bokretsion G. Brhane[1], Hassen Mamo[4], Beyene Petros[4], Hiwot Solomon[5], Ebba Abate[1], Chris Hennelly[2], Madeline Denton[6], Steven R. Meshnick[2], Jonathan J. Juliano[2], Jeffrey A. Bailey[3], Jane Cunningham[7], **Jonathan B. Parr**[2]

[1]*Ethiopian Public Health Institute, Addis Ababa, Ethiopia,* [2]*University of North Carolina, Chapel Hill, NC, United States,* [3]*Brown University, Providence, RI, United States,* [4]*Addis Ababa University, Addis Ababa, Ethiopia,* [5]*Federal Ministry of Health, Addis Ababa, Ethiopia,* [6]*University of Florida, Gainesville, FL, United States,* [7]*World Health Organization, Geneva, Switzerland*

Plasmodium falciparum parasites with deletions of the *histidine-rich protein* 2 and 3 (*pfhrp2/3)* genes may not be detected by commonly deployed malaria rapid diagnostic tests (RDTs) in Africa. Because RDTs inform the majority of malaria diagnosis in Ethiopia, reports of these deletions in neighboring countries have raised concern that existing diagnostic strategies are threatened. To determine the impact of these parasites in Ethiopia, we conducted the first study using the World Health Organization's (WHO's) protocol for *pfhrp2/3*-deleted parasite surveillance and used a novel deep sequencing approach to confirm gene deletions. 12,572 subjects with suspected malaria who presented to government health facilities in the Amhara, Tigray, and Gambella regions were enrolled and underwent testing with two distinct, WHO pre-qualified RDTs. *P. falciparum* infection was confirmed in 2,704 (22%) subjects by ≥1 RDT, of which 350 (13%) were flagged as potential *pfhrp2/3* deletions due to discordant RDT results (i.e. PfHRP2-, Pf-pLDH+). Molecular testing of 176/350 (50.2%) samples with the discordant RDT profile of interest and sufficient parasite densities for *pfhrp2/3* genotyping confirmed that 113 (64%) were *pfhrp2-/3-*, with an additional 43 (24%) *pfhrp2-/3+* or *pfhrp2+/3-*. We confirmed the absence of PfHRP2 antigenemia in 129 of 169 (76%) samples with the discordant RDT profile using a Luminex bead-based immunoassay. Molecular inversion probe (MIP) and whole-genome sequencing confirmed the presence of gene deletions and enabled high-resolution mapping of deletion regions for 271 subjects with *P. falciparum* malaria. MIP results revealed distinct deletion breakpoint profiles into which samples could be categorized; analyses are underway to identify clinical associations. In summary, large-scale surveillance near Ethiopia's border with Eritrea and Sudan confirmed the presence of *pfhrp2/3*-deleted parasites in all surveyed regions by a multi-omics diagnostic approach. These findings provide strong evidence that a change in malaria diagnostic strategy needs to be considered as per WHO guidelines.

484

METABOLOMICS BIOMARKER DISCOVERY IN SEPSIS PATIENTS FROM AUSTERE ENVIRONMENTS

Joost Brandsma[1], Deborah Striegel[1], Will Thompson[2], Paul Blair[1], Josh Chenoweth[1], Subramaniam Krishnan[1], Kevin Schully[3], Lisa St. John-Williams[2], George Oduro[4], Daniel Ansong[4], Andrew Letizia[5], Anne Fox[5], Marvin Sklar[6], Charmagne Beckett[7], Benjamin Espinosa[3], Ephraim Tsalik[2], Christopher Woods[2], Danielle Clark[1]

[1]*Henry M. Jackson Foundation, Austere Environments Consortium for Enhanced Sepsis Outcomes, Bethesda, MD, United States,* [2]*Duke University, School of Medicine, Durham, NC, United States,* [3]*Biological Defence Research Directorate, Naval Medical Research Center-Frederick, Ft. Detrick, MD, United States,* [4]*Komfo Anokye Teaching Hospital, Kumasi, Ghana,* [5]*Naval Medical Research Unit-3 Ghana Detachment, Accra, Ghana,* [6]*Naval Medical Research Center - Asia, Singapore, Singapore,* [7]*Infectious Diseases Directorate, Naval Medical Research Center, Silver Spring, MD, United States*

Expeditious and accurate information is critical for clinical decision-making in sepsis and can be enhanced using host-response biomarkers indicative of sepsis type and prognosis. The Austere Environments Consortium for Enhanced Sepsis Outcomes (ACESO) is a consortium of military medical and academic research institutes aiming to improve early recognition, diagnosis and treatment of sepsis in low-resource settings. Our aim was to stratify patients enrolled in an ACESO observational study based on their blood metabolic phenotype and identify sets of host-biomarkers able to predict outcomes in each phenotype. Sepsis patients were recruited at clinical sites in Cambodia, Ghana and the USA (n=552), and concentrations of 150 endogenous blood plasma metabolites were quantified using the Biocrates AbsoluteIDQ p180 Kit. Topological Data Analysis (TDA) was used to cluster patients according to similarities in their plasma metabolite profile. Significance of between-cluster differences and trends in metabolite levels, as well as patient metadata (demographics, clinical, laboratory) was assessed using non-parametric tests. The TDA of the plasma metabolome yielded three major sepsis phenotypes, each characterized by a distinct plasma metabolite profile. Membership of each phenotype was associated with statistically significantly differences in outcome, with 28-day mortality ranging from 0-17% to 20-58%, depending on recruitment site. In addition, we found significant differences in age, clinical presentation (physical examination, vital signs), laboratory measurements (including cell counts), number of ICU admissions, and 12-month mortality, but not pathogen category (confirmed bacterial or viral infection). Highest mortality was strongly associated with elevated short-chain acylcarnitine and decreased sphingomyelin levels, as well as a decreased lyso-to-diacyl-phosphatidylcholine ratio. Further studies into the dysregulation of these metabolic pathways and their role in sepsis pathogenesis and outcomes are warranted.

485

PERINATAL TRANSMISSION OF ANTIMICROBIAL RESISTANT ORGANISMS - BANGLADESH

Ashley Styczynski[1], Md. Badrul Amin[2], Shahana Parveen[3], Md. Abu Pervez[4], Dilruba Zeba[4], Emily Gurley[5], Stephen Luby[1]

[1]*Stanford University, Palo Alto, CA, United States,* [2]*icddr,b, Dhaka, Bangladesh,* [3]*icddrb, Dhaka, Bangladesh,* [4]*Faridpur Medical College Hospital, Faridpur, Bangladesh,* [5]*Johns Hopkins University, Baltimore, MD, United States*

Antimicrobial resistance (AMR) is a global health threat that disproportionately affects LMICs and leads to substantial neonatal mortality. An ongoing study of childhood mortality in Bangladesh revealed a common cause of death among neonates is sepsis from Gram-negative AMR organisms. To ascertain factors leading to neonatal exposure, we enrolled 100 women presenting for delivery to Faridpur Hospital during February-March 2020. We collected vaginal and rectal swabs from mothers on presentation and at least 24 hours after delivery as well as rectal swabs from newborns. Swabs were plated on chromogenic agars selective for extended-spectrum-beta-lactamase-(ESBL) producing organisms and carbapenem-resistant Enterobacteriaceae (CRE). Eighty-five percent of women underwent C-section. Prior to delivery, ESBL organisms were isolated from 15% of vaginal and 63% of rectal swabs. CRE was detected in 2% of vaginal and 8% of rectal swabs. Following delivery, colonization exceeded 90% and 70%, respectively, in both swab sets. Among newborns, 85% were colonized with ESBL and 67% with CRE. Maternal AMR colonization on admission did not correlate with income, education, parity, prenatal care, or prior antibiotic use. However, rectal CRE colonization correlated with hospitalization during pregnancy (OR 11.9, p<0.01). Maternal colonization at discharge was associated with labor management practices and delivery mode. Membrane stripping was predictive of vaginal ESBL (OR 9.0, p<0.01), rectal CRE (OR 5.0, p=0.03), and vaginal CRE (OR 2.9, p=0.09) colonization; C-section was significantly associated with colonization across all sites (OR 4.0-15.4, p<0.05). Among newborns delivered by C-section, there was an 8-9-fold increased risk of ESBL and CRE colonization (p<0.01). These results strongly suggest that AMR is horizontally transmitted in the perinatal setting, and invasive procedures increase risk of AMR colonization, which could lead to the development of untreatable infections. These findings emphasize the urgent need for enhanced infection prevention and control practices to preserve the benefits of hospital-based deliveries.

486

ALARMING INCIDENCE OF NEONATAL SEPSIS AND ANTIMICROBIAL RESISTANCE AT TWO LARGE HOSPITALS IN ETHIOPIA

John Cranmer[1], Abebe Gobezayehu[1], Lamesgin Alamineh[2], Gizachew Yismaw[3], Joseph Hopkins[4], Mulusew Belew[2], Habib Yakubu[5], Lindsay Denny[5], **Christine L. Moe**[5]

[1]*Emory University-Nell Hodgson Woodruff School of Nursing, Atlanta, GA, United States,* [2]*Emory Ethiopia, Amhara Regional Office, Bahir Dar, Ethiopia,* [3]*Amhara Public Health Institute, Bahir Dar, Ethiopia,* [4]*Emory University, Atlanta, GA, United States,* [5]*Emory University-Rollins School of Public Health, Atlanta, GA, United States*

As child mortality has declined globally, an ever-increasing proportion of child deaths occur during the newborn period. Globally, 25% of newborns die from sepsis, and 75% of newborn deaths in Ethiopia occur within the first 7 days of life. Little is known about the risks of sepsis acquisition and antimicrobial resistance (AMR) among newborns receiving care at health facilities. In Ethiopia, we studied the relationship between water, sanitation, and hygiene conditions (WASH), environmental contamination, and incident sepsis/AMR at two large obstetric hospitals. This sepsis study recruited normal (NBW) and low birth weight (LBW) infants (<2000g) from three clinical units (NICUs, KMC, post-natal) at two hospitals (one general (DT) and one referral (FH) hospital) with contrasting WASH and environmental conditions in Amhara, Ethiopia. All LBW and every 5th NBW infant were recruited at birth. Blood samples from infants with clinically suspected sepsis were cultured and tested for AMR. We recruited 615 infants: 81% were NBW; 33% had suspected clinical sepsis; 22% had sepsis confirmed by culture. Of 576 infants with data through 7 days of life, 5% deceased (early neonatal mortality ratio=46.9. NMR). 59% of deaths were due to sepsis, followed by prematurity (22%) and respiratory distress (15%). 133 blood cultures were positive for 21 organisms. The primary isolates were *S. aureus* (29%) and *Klebsiella* (29%), but organism dominance differed by hospital. DT sepsis cases primarily had *S. aureus* (41%) versus *Klebsiella* (39%) at FH. 87% of isolates were resistant to one or more of 14 antibiotics. 32% were resistant to 1st-line empiric sepsis treatment. Only 3% of organisms resistant to 1st-line treatment were susceptible to 2nd line drugs. We found high neonatal sepsis incidence and antimicrobial resistance at two large hospitals in Ethiopia. The 7-day NMR of 46.9 far exceeds the 28-day benchmarks of 27.2 for sub-Saharan Africa and 30 for Ethiopia. Pervasive antimicrobial resistance—particularly to 1st and 2nd line empiric treatment—coupled with high sepsis mortality presents an urgent call to prevent sepsis in health facilities globally.

487

ASSESSMENT OF BACTERIAL ETIOLOGY, ANTIMICROBIAL RESISTANCE AND RISK FACTORS FOR NEONATAL SEPSIS IN A NEONATAL INTENSIVE CARE UNIT (NICU) OF A TERTIARY CARE HOSPITAL IN NEPAL: A PROSPECTIVE COHORT STUDY

Sulochana Manandhar[1], Puja Amatya[2], Imran Ansari[2], Neeva Joshi[1], Nhukesh Maharjan[1], Sabina Dongol[1], Buddha Basnyat[1], Sameer Dixit[3], Stephen Baker[4], Abhilasha Karkey[1]

[1]*Oxford university clinical research unit, Patan Academy of Health Sciences, Kathmandu, Nepal,* [2]*Department of paediatrics, Patan Academy of Health Sciences, Kathmandu, Nepal,* [3]*Center for molecular dynamics Nepal, Kathmandu, Nepal,* [4]*Cambridge Infectious Diseases, University of Cambridge, Cambridge, United Kingdom*

Sepsis is an overwhelming and life threatening response to bacteria in the bloodstream and a major cause of neonatal morbidity and mortality. Understanding the etiology and potential risk factors, especially for neonates, is urgently required, particularly in low-income countries where burden of pathogens is high and epidemiology is poorly understood. This prospective cohort study was conducted in a level three NICU of a tertiary care hospital in Nepal to determine the incidence rates, potential risk factors, etiology and antimicrobial resistance (AMR) associated with neonatal sepsis. Among 142 NICU admitted neonates enrolled from April 2016 to October 2017, incidence of blood culture-positive sepsis was 15% (21/142). Some 86% (38/44) of sepsis were hospital acquired and 89% (39/44) of isolates were Gram-negative bacilli with 72% (31/43) being multidrug resistant (MDR). *Klebsiella pneumoniae* (34%, 15/44) was the commonest isolate followed by *Enterobacter* spp. (25%, 11/44) and *Acinetobacter* spp. (18%, 8/44). The AMR to ampicillin (100%, 43/43), cefotaxime (74%, 31/42) and ampicillin-sulbactam (55%, 21/38) were the highest with bla_{TEM} (53%, 18/34) and bla_{KPC}(46%, 13/28) being the commonest AMR genes. When comparing culture positive sepsis to the non-sepsis group: the use of invasive intravenous lines, delayed feeding, and length of NICU stay were significantly ($p < 0.05$) prolonged in the culture proven sepsis group. Leukopenia (<7,000 WBC/μL), thrombocytopenia (<150,000 platelets//μL) and elevated CRP (>6 mg/dl) were significantly higher in culture positive sepsis.With 80% of neonatal sepsis episodes being hospital acquired, our study indicated various associated nosocomial risk factors and underscored the need to improve local infection control measures, so as to reduce the existing burden of sepsis. We highlight certain sepsis associated laboratory parameters along with identification of resistance genes among the bacterial etiology, which can act as a guide for early and better therapeutic management of sepsis. These findings could be extrapolated to other low-income settings within the region.

488

HYPERVIRULENT MULTIDRUG-RESISTANT *KLEBSIELLA* SPP. CAUSING SEVERE AND FATAL DISEASE IN CHILDREN IN RURAL MOZAMBIQUE

Arsenia Joana Massinga[1], Augusto Messa Júnior[1], Nélio Nobela[1], Marcelino Garrine[1], Sergio Massora[1], Anélsio Cossa[1], Delfino Vubil[1], Hélio Mucavel[1], Tacilta Nhampossa[1], Clara Menendez[1], Clara Menendez[2], Robert Breiman[3], Dianna Blau[4], Quique Bassat[2], Inácio Mandomando[1]

[1]*Centro de Investigação em Saúde de Manhiça, Manhiça, Mozambique,* [2]*ISGlobal, Hospital Clínic - Universitat de Barcelona, Barcelona, Spain,* [3]*Emory Global Health Institute, Emory University, Atlanta, GA, United States,* [4]*Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, United States*

Klebsiella spp. are important pathogens associated with severe disease among young children admitted to Manhiça District Hospital (MDH), Mozambique. *K. pneumoniae* have been shown, in postmortem studies in Manhiça, to be important pathogens leading to death of children under 5 years of age, supporting a larger role than previously considered in childhood mortality. We characterized the virulence repertoire, antimicrobial susceptibility profile and resistance mechanisms of *Klebsiella* spp. isolates from blood of children hospitalized at MDH (n=84) and from children under-5 who died and were part of the undergoing Child Health and Mortality Prevention Surveillance (CHAMPS) study (n=21). Antimicrobial susceptibility, associated mechanisms and virulence factors were assessed by disk diffusion, multiplex Polymerase Chain Reaction (PCR) and sequencing. *Klebsiella* isolates from CHAMPS cases were frequently multidrug resistant but not significantly different from those obtained from bacteremic children (66.7%, 14/21 *vs*. 64.3%, 54/84). Postmortem isolates were more likely to be resistant to ceftriaxone (66.7%, 14/21 *vs.* 46.4%, 39/84, p=0.027) and more commonly harbored extended-spectrum β-lactamases (57.1%, 12/21 *vs.* 22.6%, 19/84 p=0.002) than bacteremic ones, with $bla_{CTX\text{-}M\text{-}15}$ accounting for 57.1% (8/14) and 28.2% (11/39), of ceftriaxone-resistant postmortem and bacteremic isolates, respectively p=0.053. Postmortem isolates were significantly more associated with the presence of hypervirulent genes than those from bacteremic children: *magA* (38.1% *vs.* 8.3%, p=0.002), *rmpA* (33.3% *vs.* 9.5%, p=0.011) and *traT* (66.6%, 14/21 *vs.* 10.7%, 9/84 p<0.0001). Interestingly, isolates from the bacteremic children who died were more likely to be resistant to chloramphenicol compared to survivors (60.9%, 14/23 *vs.* 38.1%, 16/42, p=0.109) and harboring *hylC* (17.4%, 4/23 *vs*. 4.8%, 2/42) and *sat* (21.7%, 5/23 *vs.* 9.5%, 4/42) (statistically non-significant). Multidrug-resistant hypervirulent *Klebsiella* spp. has emerged in Mozambique, associated with poor outcomes, highlighting needs for, prompt diagnosis, prevention and control.

489

RARE ORGANISMS IDENTIFIED THROUGH MITS IN BANGLADESH: POTENTIAL CONTRIBUTION IN CHILD DEATH?

Mustafizur Rahman[1], Dilruba Ahmed[1], Muntasir Alam[1], M Ishrat Jahan[1], Afruna Rahman[1], Farzana Islam[1], Kyu Han Lee[2], Shafina Jahan[1], Sanwarul Bari[1], Emily S. Gurley[2], Shams El Arifeen[1]

[1]*icddr,b, Dhaka, Bangladesh,* [2]*Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States*

In Low- and Middle-Income Countries (LMIC) laboratory diagnosis of clinical specimens is largely dependent on classical microbiological methods. The Child Health and Mortality Prevention Surveillance (CHAMPS) Network uses an automated blood culture and identification platform and molecular techniques along with pathologist examination to identify infectious agents from post-mortem specimens from stillbirths and children under-5 years. A number of pathogens detected through these methods were previously unreported from Bangladeshi populations. Hence, these pathogens may be overlooked in terms of their potential to cause child death in Bangladesh and elsewhere. The aim of the current work is to analyze their possible contribution to child death. From October 2017 - October 2019, post-mortem specimens were investigated from 126 cases and infectious agents were identified from 93 (74% of 126) by both microbial culture or molecular technique. We identified 28 rare infectious agents from 34 deaths, including 11 stillbirths, 14 early neonates (<7 days), 1 late neonate (7-28 days) and 2 children. Among these pathogens,19 were bacteria (11 Gram negative and 8 Gram positive), 5 were viruses, 3 were fungi and 1 was a parasite. These rare isolates were detected by either microbial culture or Taqman array card platform based real-time polymerase chain reaction. Some of them were previously reported be resistant to antibiotics and disinfectants such as S*tenotrophomonas maltophilia, Enterobacter cloacae, Burkholderia cepacia, Staphylococcus lugdunensis* and many were reported as premature labor associated bacteria such as *Ochrobactrum anthropi, Chlamydia trachomatis, Orientia tsutsugamushi, Rothia dentocariosa, Streptococcus mitis, and Kocuria kristinae*. Special emphasis should be given to explore their contribution to the burden of under-5 child illness and death in Bangladesh.

490

CHANGE IN *SALMONELLA* TYPHI INCIDENCE AND ANTIMICROBIAL RESISTANCE PATTERNS FOLLOWING MASS VACCINATION WITH THE NEW TYPHOID CONJUGATE VACCINE

Ioana Diana Olaru[1], Nicholas Feasey[2], Rashida A. Ferrand[1], Janice A. Martin[3], David Mabey[1], Heidi Hopkins[1], Sekesai Mtapuri-Zinyowera[4], Prosper Chonzi[5], Katharina Kranzer[1]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*Liverpool School of Tropical medicine, Liverpool, United Kingdom,* [3]*Biomedical Research and Training Institute, Harare, Zimbabwe,* [4]*National Microbiology Reference Laboratory, Harare, Zimbabwe,* [5]*Department of Health - Harare City, Harare, Zimbabwe*

Salmonella Typhi with diminished ciprofloxacin susceptibility (DCS) has recently emerged in sub-Saharan Africa. To control an ongoing outbreak of typhoid in Harare, a mass vaccination campaign with the new typhoid Vi-conjugate vaccine (TCV) was conducted in March 2019. All children aged 6 months to 15 years were vaccinated with an administrative coverage exceeding 85%. Here we describe the impact of TCV mass vaccination on *S.* Typhi cases and antimicrobial resistance (AMR) in Harare. These data were collected as part of a multi-centre febrile illness aetiology study (www.lshtm.ac.uk/fiebre). One blood culture per patient was collected. Antimicrobial susceptibility testing was performed by disc-diffusion and E-tests using EUCAST breakpoints. Multidrug resistance (MDR) was defined as resistance to ampicillin, chloramphenicol and co-trimoxazole. *S.* Typhi was isolated from 129**/**565 blood cultures (22.8%) from children and adults. Of those, 41 (32%) were positive before-, 82 (64%) after- and 6 (4%) during the month of the mass vaccination campaign. There was a decrease in the proportion of children with *S.* Typhi isolated from blood culture following the vaccination campaign, from 23/104 (22%) to 8/82 (9.8%, p=0.025). Ciprofloxacin resistance as determined using the pefloxacin disc fell from 34/41 (83%) before to 44/82 (54%, p=0.001) after the vaccination campaign mostly driven by a change in prevalence in children. Minimum inhibitory concentration (MIC) data for ciprofloxacin was available for 86 isolates. DCS (MICs 0.06 to 1 µg/mL) was present in 60 (70%) of isolates. There was a trend toward lower MICs for ciprofloxacin after the campaign (p=0.003). The prevalence of MDR was unchanged at 88% pre- and post-campaign. These data show a decrease in the prevalence of DCS following the TCV mass vaccination campaign. This may be due to a localized point-source outbreak associated with a DCS sub-lineage 4.3.1 that was ended by vaccination. Although it reduced the proportion of febrile children with typhoid fever, the high number of cases identified post-vaccination campaign emphasizes the need for further control measures to curb this outbreak.

491

EBOLA ASSOCIATED HEARING LOSS

Samuel C. Ficenec[1], Donald Grant[2], Michael Gbakie[2], Ibrahim Sumah[2], Susan D. Emmett[3], John S. Schieffelin[1]

[1]*Tulane School of Medicine, New Orleans, LA, United States,* [2]*Tulane Viral Hemorrhagic Fever Organization, Kenema, Sierra Leone,* [3]*Duke University School of Medicine, Durham, NC, United States*

Hearing loss is the second leading cause of disability affecting approximately 19% of the worlds population. Despite well known social, economic, and neurologic consequences this condition receives little attention. Ebola (EBV) has been noted to cause hearing loss. However, the true burden of this sequelae is likely underestimated due to a lack of standardized measurement and reporting. This was a cross-sectional study of EBV survivors and household controls. Upon recruitment survivors and controls were screened for hearing loss by determining Pure Tone Averages (PTA) of air conduction thresholds using an AMBCO audiometer, according to WHO standards. Individuals found to have elevated PTAs were referred to confirmatory testing measuring both air and bone conduction using a SHOEBOX audiometer to differentiate between sensorineural and conductive hearing loss. Additionally, all subjects completed symptom questionnaires and physical exams to understand the full spectrum of viral sequelae. 301 EBV survivors and 711 controls were recruited for this study. The average age of EBV survivors was higher than household controls (30.2 vs 22.4, p<0.001). Of 301 EBV survivors, 70 (23.2%) were found to have hearing loss in comparison to 67 (9%) controls. Logistic regression found that risk factors associated with EBV related hearing loss are symptoms and signs affecting the middle and inner ear (OR 3.6, p<0.001), the eye (OR 2.6, p<0.001), and the nervous system (OR 2.1, p=0.026). Interestingly, pulmonary complaints were protective of hearing loss (OR 0.49, p=0.033). These results clearly demonstrate a profound relationship between hearing loss EBV. This study further characterizes the sequelae resulting from EBV and suggest possible mechanisms of their development. Due to the profound effects of untreated hearing loss it is imperative that greater emphasis be placed on understanding EBV related hearing loss to decrease the burden of this impairment and improve the quality of life among survivors.

492

POST-EBOLA SYNDROME PRESENTS WITH MULTIPLE OVERLAPPING SYMPTOM CLUSTERS: EVIDENCE FROM AN ONGOING COHORT STUDY IN EASTERN SIERRA LEONE

Nell G. Bond[1], Emily Engel[1], Lansana Kanneh[2], Robert Samuels[3], Adaora Okoli[1], Sarah T. Himmelfarb[1], Jeffrey Shaffer[1], Donald Grant[2], John Schieffelin[1]

[1]*Tulane University SOM, New Orleans, LA, United States,* [2]*Kenema Government Hospital, Kenema, Sierra Leone,* [3]*Vanderbilt University, Nashville, TN, United States*

Following the 2013-2016 West African Ebola outbreak, a large cohort of Ebola survivors with distinct, persistent health complaints was recognized. Here we provide an in-depth characterization of post-Ebola syndrome in survivors 2.6 years after resolution of disease. Additionally, we report sub-phenotypes of post-Ebola syndrome with overlapping symptom clusters in survivors sampled in Eastern Sierra Leone. Potential survivor participants in Eastern Sierra Leone were identified by the Sierra Leone Association of Ebola Survivors. Household contacts were identified by enrolled survivors. EVD survivors and their contacts were administered a questionnaire assessing self-reported symptoms and a physical exam. Symptoms were then compared using hierarchical clustering. Both SPICE and correlation analyses were performed to explore the relationships between symptom clusters. Statistical analysis was conducted using conditional logistic regression. Between March 2016 and January 2019, 375 Ebola survivors and 1040 contacts were enrolled into the study. At enrollment, Ebola survivors of all age groups reported significantly more symptoms than their contacts in all categories. Six symptom clusters were identified representing distinct organ systems. SPICE revealed 2 general phenotypes: with or without rheumatologic symptoms. Clusters including rheumatologic symptoms were correlated with one another (r=0.63) but not with other clusters (r<0.35). Ophthalmologic/auditory symptoms were moderately correlated with the non-rheumatologic clusters (r>0.5). Interestingly, psychologic/neurologic, cardiac/GI and constitutional clusters correlated with one another (r>0.6). p<.0001 in all cases. This study presents an in-depth characterization of post-Ebola syndrome in Sierra Leonean survivors 2.6 years after disease. The interrelationship between symptom clusters indicates that post-Ebola syndrome is not a homogenous disease. The distinct phenotypes identified likely require targeted therapies to optimize treatment for EVD survivors long-term.

493

IMPACT OF ORAL ANTIMALARIAL TREATMENT ON MORTALITY IN PATIENTS WITH EBOLA VIRUS DISEASE: A MULTISITE COHORT STUDY

Logan Abel[1], Stephanie Chow Garbern[2], Tao Liu[3], Derrick Yam[3], Shiromi Perera[4], Stephen Kennedy[5], Moses Massaquoi[5], Foday Sahr[6], Adam C. Levine[2], Adam R. Aluisio[2]

[1]*Warren Alpert Medical School of Brown University, Providence, RI, United States,* [2]*Department of Emergency Medicine, Brown University Alpert Medical School, Providence, RI, United States,* [3]*Brown University, School of Public Health, Center for Statistical Sciences, Department of Biostatistics, Providence, USA, Providence, RI, United States,* [4]*International Medical Corps, Los Angeles, CA, United States,* [5]*Ministry of Health, Monrovia, Liberia,* [6]*College of Medicine and Allied Health Sciences, University of Sierra Leone, Freetown, Sierra Leone*

At the present, protocol-based management of Ebola virus disease (EVD) recommends empiric malaria treatment, however there is limited evidence on patient-centered benefits with that approach. This study evaluated the association between early administration of oral antimalarial medication and mortality among patients with EVD in outbreak settings. This retrospective cohort study accrued patients with EVD admitted to five International Medical Corps operated Ebola Treatment Units (ETU) in Sierra Leone and Liberia during 2014-2015. The antimalarial administration protocol was empiric oral treatment with four tablets of combination artemether and lumefantrine, twice a day for three days, however, due to resource variability, only a subset of patients received treatment. Data on sociodemographics, clinical characteristics, malaria rapid diagnostic test results and Ebola viral loads (cycle threshold [CT] values) were collected. The outcome of interest was mortality compared between patients treated with oral antimalarials initiated within 48-hours of admission to those not treated. Logistic regression was used to yield adjusted odd ratios (aOR) with associated 95% confidence intervals (CI). Multivariable analyses controlled for ETU location, malaria status, age, CT value, symptoms of bleeding, diarrhea, dysphagia and dyspnea, and additional supportive treatments. Overall, there were 424 cases analyzed, among which 376 (88.7%) received oral antimalarials within 48 hours of admission. The median age was 30 years (Interquartile Range [IQR]: 16, 44) and 59.7% were female. The most common symptoms were diarrhea (85.6%) and anorexia (80.7%). Overall mortality occurred in 57.5% of cases. In antimalarial treated cases unadjusted mortality prevalence was 55.1% versus 77.1% for those untreated (p=0.005). Multivariable analysis demonstrated significantly reduced odds for mortality with oral antimalarial treatment versus non-treatment (aOR=0.34, 95% CI: 0.12, 0.92, p=0.039). Given the current data, empiric antimalarial provision in EVD care is likely warranted however further study in epidemic settings is needed.

494

LASSA FEVER AMONG CHILDREN IN EASTERN PROVINCE, SIERRA LEONE: A 7-YEAR RETROSPECTIVE ANALYSIS (2012-2018)

Robert J. Samuels[1], Donald S. Grant[1], Joseph R. Starnes[2], Emily Engel[3], Jeffrey G. Shaffer[3], John S. Schieffelin[3], Troy D. Moon[2]

[1]*Kenema Government Hospital, Kenema, Sierra Leone,* [2]*Vanderbilt Institute for Global Health, Nashville, TN, United States,* [3]*Tulane University, New Orleans, LA, United States*

Sierra Leone is considered hyperendemic for Lassa Fever (LF). There are few studies to date on LF in children, making it difficult to fully understand best practices for pediatric management. LF presentation can be indistinguishable from other viral hemorrhagic fevers (VHFs) or from other febrile illnesses such as malaria and typhoid fever. The 2013-2015 West African Ebola outbreak and recent outbreak in Democratic Republic of Congo, have brought new advances in the clinical management of Ebola, as well as renewed interest in understanding the management of other VHFs, such as LF. We aim to describe the clinical characteristics of hospitalized pediatric patients, either suspected or confirmed LF, and to assess factors associated with hospital outcomes among antigen positive pediatric LF patients. A retrospective cohort study was conducted using routine data from the LF ward at Kenema Government Hospital in Kenema, Sierra Leone. All children less than 18 years old that were LF antigen positive or suspect cases upon admission between January 2012 and December 2018 were included in this analysis. A total of 292 children were evaluated for possible LF. Most (79%) of the LF suspects/confirmed children were admitted in the three-year period from 2012-2014. Antigen-positive children were more likely to be male (63% vs. 47%; p=0.031) and were more likely to die (63% vs. 11%; p<0.01). Overall, mortality was high (21%). Among antigen-positive children, those that will eventually die were more likely to have symptoms of bleeding (p=0.033), confusion (p=0.012) as well as significantly elevated serum creatinine (p=0.004), alanine aminotransferase (p=0.001), and potassium (p=0.003) levels. Treatment with Ribavirin was not found to be significantly associated with survival (p=0.916). These data provide insights into the current pediatric LF presentations and management. A high index of suspicion and patient monitoring are needed for management of these cases in endemic areas. More research in creating predictive algorithms around antigen-positivity and hospital outcomes is needed in the management of LF and, broadly, other VHFs.

495

A MATCHED COHORT STUDY TO CHARACTERISE THE CLINICAL MANIFESTATIONS OF DENGUE IN PREGNANCY AND INVESTIGATE THE SPECTRUM OF ADVERSE MATERNAL AND FETAL OUTCOMES

Nguyet M. Nguyen

Oxford University Clinical Research Unit, Ho Chi Minh, Viet Nam

Dengue virus (DENV) is a major arbovirus, which is endemic in tropical and subtropical areas and cause the most common disease in humans. Although Zika virus (ZIKV) is a closely related flavivirus with DENV and is recognized to cause major adverse effects on fetal development and infant outcomes, there is limited data on the effects of dengue on pregnant women or their babies. We performed a prospective study at the Hospital for Tropical Diseases, HCMC, enrolling all pregnant women with suspected dengue, together with 1-2 non-pregnant controls matched by maternal age and day of illness at recruitment. In total, 212 pregnant women and 329 non-pregnant controls, hospitalized between Oct16 and Jan18, were confirmed to have dengue. Among pregnant patients, 57 (27%) got dengue in the first trimester, 99 (47%) in the second and 56 (26%) in the third. The admission threshold was lower for the pregnant women than the controls (42% had one or more warning signs at admission versus 60%, respectively). Despite this, similar proportions in each group developed severe vascular leakage (1% versus 2% in pregnant and control groups, respectively), resulting in shock and/or respiratory distress. No patient developed severe bleeding and all women recovered fully. A detailed description of the clinical and laboratory evolution of acute dengue in two groups and a dynamic model predicting risk of severe disease progress will be presented. Pregnancy outcome data were available for 200/212 pregnant women: 11/57 (19%) of women infected in the first trimester had vaginal bleeding and progressed to miscarriage thereafter; hypertension and diabetes developed in only a small number; among the 189 live births, 9 were born before the 37th week, 6 had low birth weight (<2500g), and 18 were admitted to NICU including several infants with congenital anomalies. There were no neonatal deaths. The infants are now in a follow up study to assess neurodevelopment during the first 2 years of life. We have demonstrated a number of serious adverse maternal and fetal outcomes associated with dengue in pregnancy, indicating that this may be a significant public health concern in endemic settings.

496

LEVERAGING BIOMARKERS OF EXPOSURE IDENTIFIED IN PAGODAS (PEDIATRIC ASSESSMENT GROUP OF DENGUE AND *AEDES* SALIVA) IN CAMBODIA

Fabiano Luiz Oliveira[1], James Oristian[1], Dara Kong[2], Daniel M. Parker[3], Paola Valenzuela Leon[1], Brook Jeang[3], Somnang Man[4], Sokunthea Sreng[4], Seila Suon[4], Rekol Huy[4], Rithea Leang[4], Jennifer A. Bohl[2], Eric Calvo[1], Jesus G. Valenzuela[1], Jessica E. Manning[5]

[1]*Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States,* [2]*Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Phnom Penh, Cambodia,* [3]*Department of Population Health and Disease Prevention, University of California, Irvine, CA, United States,* [4]*National Center of Parasitology, Entomology, and Malaria Control, Phnom Penh, Cambodia,* [5]*National Institute of Allergy and Infectious Diseases, Phnom Penh, Cambodia*

Dengue virus is a serious public health concern worldwide. During blood feeding, mosquitos deposit salivary proteins into the dermis that elicit an antibody response. Defining the burden of dengue disease and the *Aedes aegypti* vector determinants that exacerbate disease transmission are critical for Cambodian public health authorities with limited vector control tools. In this longitudinal pediatric cohort, we enrolled 771 healthy Cambodian children aged 2 to 9 years old in July 2018 to be followed twice per year (wet and dry seasons) for serosurveillance of *Ae. aegypti* exposure by measuring the antibody response to mosquito salivary proteins. Immunoblotting of *Ae. aegypti* salivary gland homogenate identified five immunodominant *Aedes* salivary proteins based on molecular weight. We produced five corresponding recombinant *Ae. aegypti* salivary proteins in 293F cells to: 1) validate the original salivary gland homogenate antibody intensity and 2) use as biomarkers of mosquito exposure in our cohort. Traditional entomological surveys were also performed semi-annually to correspond with the serosurveillance. As the recombinant protein validation is still ongoing, *Ae. aegypti* Apyrase and D7s recombinant salivary proteins arise as the most immunogenic. Further analyses will demonstrate temporal variation of whole salivary gland homogenate antibody intensity from wet to dry season over 2 years, magnitude of difference from dengue-negative versus acute dengue cases versus asyptomatic dengue cases as of October 2020; and correlations (or lack thereof) of each recombinant marker with *Ae. aegypti* anti-saliva antibody intensity at wet and dry seasons. Geospatial analyses will compare the signal of recombinant markers with mean larval density and Premises Condition Index (PCI) scoring in specific targeted areas. Our preliminary findings have identified five immunodominant *Ae. aegypti* salivary proteins that may serve as biomarkers of mosquito exposure. By the time of presentation, we will also assess their relationship to whole salivary gland homogenate antibody intensity, disease status, and traditional entomological indices.

497

CHALLENGES AND OPPORTUNITIES UTILIZING AN EXISTING RESEARCH NETWORK FOR NEW PROTOCOLS DURING THE COVID-19 PANDEMIC: THE SPECIAL PATHOGENS RESEARCH NETWORK EXPERIENCE

Mark Kortepeter[1], LuAnn Larson[1], Lindsay J. Hicks[1], Bruce Gordon[1], Abbey Lowe[1], Christa Arguinchona[2], Henry Arguinchona[2], Nahid Bhadelia[3], Theodore J. Cieslak[1], Richard Davey[4], Kerry Dierberg[5], Jared D. Evans[6], Maria G. Frank[7], Jonathan Grein[8], Andre C. Kalil[1], Susan Kline[9], Colleen S. Kraft[10], Christopher J. Kratochvil[1], Susan McLellan[11], Aneesh K. Mehta[10], Brooke Noren[9], Vanessa Raabe[5], Shelly Schwedhelm[1], Erica S. Shenoy[12], Timothy Uyeki[13], Sami Vasistha[1], Lauren Sauer[14]

[1]*University of Nebraska Medical Center, Omaha, NE, United States,* [2]*Providence Medical Center, Spokane, WA, United States,* [3]*Boston University Medical Center, Boston, MA, United States,* [4]*National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States,* [5]*Bellevue Hospital, New York City, NY, United States,* [6]*Applied Physics Lab, Johns Hopkins University, Laurel, MD, United States,* [7]*Denver Health Medical Center, Denver, CO, United States,* [8]*Cedars Sinai Hospital, Los Angeles, CA, United States,* [9]*University of Minnesota Medical Center, Minneapolis, MN, United States,* [10]*Emory University Hospital, Atlanta, GA, United States,* [11]*University of Texas Medical Branch, Galveston, TX, United States,* [12]*Massachusetts General Hospital, Boston, MA, United States,* [13]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [14]*Johns Hopkins University Hospital, Baltimore, MD, United States*

During the 2014-16 W. Africa outbreak of Ebola virus disease, the US had no mechanism to study investigational treatments rapidly, and individual institutions provided investigational products as emergency investigational new drugs (eINDs). Consequently, determining the optimum care for the disease was not achieved. The Special Pathogens Research Network (SPRN) was established to create the infrastructure to conduct multi-center clinical research to improve outcomes for emerging special pathogens. This included establishing a central IRB at the University of Nebraska Medical Center and 10 collaborative sites across the US. As the COVID-19 outbreak began, the SPRN quickly executed three protocols. We share efficiencies and ideas for future improvements. At the onset of the COVID-19 outbreak, the network established three clinical protocols: 1) a "natural history" protocol for collecting discarded specimens and patient data; 2) a NIAID-sponsored randomized placebo controlled trial with the antiviral drug Remdesivir; and 3) a prospective data and sample collection protocol. We evaluated the IRB approval timeline centrally and at partner sites and the rapidity of first subject enrollment. We assessed aspects that facilitated or hindered the adoption of the different protocols across the network. Central and other site IRB approvals occurred expeditiously. Subjects were enrolled within one day of site approval in all three studies. UNMC enrolled the first US patient in the Remdesivir RCT. IRB approval occurred at all 10 sites within 32 days of central IRB approval; however, contracts for data use and material transfer agreements (DUAs and MTAs) lagged. Consequently, some sites developed their own natural history protocols, rather than adopt a network protocol. In conclusion, the SPRN pre-existing network of 10 sites was able to enroll subjects rapidly at the start of the outbreak, functioning as one unit in many respects. Contracting for specimen collection protocols has proved challenging and thus has impacted the ability to organize and deliver. This continues to be addressed.

498

TAKEDA'S TETRAVALENT DENGUE VACCINE - TWO YEARS EFFICACY SURVEILLANCE

Shibadas Biswal[1], Inge Lefevre[2], Vianney Tricou[2], Martina Rauscher[2], Astrid Borkowski[2], TIDES Study Group

[1]*Takeda Vaccines, Inc, Cambridge, MA, United States,* [2]*Takeda Pharmaceuticals International AG, Zurich, Switzerland*

There is a need for a safe and efficacious vaccine against dengue infection, particularly for younger children and those who are dengue-naïve but live in dengue-endemic countries. An ongoing five-year efficacy evaluation of Takeda's tetravalent dengue vaccine (TAK-003) that started in September 2016 enrolled 20,099 healthy 4–16 year-olds living in endemic countries in Latin American (Brazil, Colombia, the Dominican Republic, Nicaragua, Panama) and Asia (the Philippines, Sri Lanka, Thailand). Children were randomized 2:1 to receive two subcutaneous injections of TAK-003 (n = 13,401) or placebo (n = 6,698) three months apart, and then monitored for febrile illness with virological confirmation of dengue (VCD) by serotype-specific RT-PCR. A subset of 4000 participants were randomly selected for additional safety, reactogenicity and immunogenicity assessments. All participants were sampled pre-vaccination and on Day 120 for dengue neutralizing antibodies, while the subset were further sampled on Days 30, 90, 270, and 450, and then annually. Such serial sampling for immunogenicity provides an opportunity to explore the effect of the vaccine on asymptomatic infections. Having previously presented the primary endpoints assessed at 12 and 18 months postvaccination, data

has now become available two years into this study for further exploratory analyses of efficacy. At two years after the second dose, 19,330 (96.2%) were still participating; 12,883 (96.1%) in the TAK-003 and 6,447 (96.3%) in the placebo groups. The vaccine was well tolerated, and rates of serious adverse events were similar in both groups. The cumulative efficacy against VCD from first dose is 72.7% (95% CI: 67.1–77.3), which includes an efficacy of 67.0% (95% CI: 53.6–76.5) in participants who were dengue-naive pre-vaccination. Efficacy against hospitalization for dengue is 89.2% (95% CI: 82.4–93.3). Further sub-group analyses, year by year analysis, and effect of the vaccine on asymptomatic infection will be presented. The trial is ongoing and continued monitoring of this population will provide data on persistence of efficacy and long-term safety of this vaccine.

499

CELL-MEDIATED IMMUNITY GENERATED BY TAKEDA'S TETRAVALENT DENGUE VACCINE CANDIDATE

Heather Friberg[1], Kristin Hatch[1], Faiza Mubashar[1], Hayden Siegfried[1], Kaitlin Victor[1], Damon Ellison[1], Richard G. Jarman[1], Shibadas Biswal[2], Derek Wallace[2], Hansi Dean[2], Vianney Tricou[2], Jeffrey R. Currier[1]

[1]*Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [2]*Takeda Vaccines, Boston, MA, United States*

A Phase IIb immunogenicity study (DEN-313) was performed in parallel with the DEN-301 Phase III efficacy study to determine the impact of baseline serostatus on the immunological performance of Takeda's tetravalent dengue vaccine candidate. Here we report on T cell responses generated out to 6 months after administration of the two-dose vaccine. PBMC were collected from 200 subjects on the day of vaccination (Day 1) and at Days 30, 120, and 270 post-vaccination, and tested by IFNγ ELISpot assay. Overlapping peptide pools matching the non-structural (NS)1, NS3, and NS5 proteins from the DENV-2 vaccine backbone as well as NS3 and NS5 from DENV-1, DENV-3, and DENV-4 were used as antigens. A subset of subjects were also tested by a Flow-ICS assay to further assess the phenotype and functional profile of antigen-specific T cells. At Day 120 (1 month post-Dose 2) IFNγ ELISpot responses were detected in 76.0% and 83.1% of baseline seropositive and seronegative subjects, respectively, and demonstrated durability for at least 6 months post-vaccination (Day 270). Peak median responses were 1952 (seropositive) and 1063 (seronegative) spot-forming cells (SFC)/million, representing 31- and 47-fold increases from baseline (Day 1). Among all subjects, 78.5% responded to DENV-2 derived peptide pools while 58.8%, 54.5%, and 45.2% responded to DENV-1, DENV-3, and DENV-4 derived peptide pools. The magnitude of responses showed a similar bias toward DENV-2 derived antigens, peaking at a median of 772 SFC/million whereas median responses to DENV-1, DENV-3, and DENV-4 peaked at 545, 392, and 330 SFC/million, respectively. NS3 was immunodominant, followed closely by NS5; NS1 elicited responses from about one-third of responders. Analysis by Flow-ICS demonstrated that T cell responses to this vaccine were predominantly mediated by CD8+ T cells, although CD4+ T cell responses were also frequently detected. DENV-specific T cells were multifunctional, producing IFNγ, TNFα, and IL-2. Overall, these data demonstrate that the vaccine was highly immunogenic and induced a potent, multifunctional, and durable T cell response independent of baseline serostatus.

501

THE OLIGOMERIC STATE OF FLAVIVIRUS E- SUBUNITS DEFINES VACCINE EFFICACY IN CHALLENGE MODELS

Stefan W. Metz, Ashlie Thomas, Devina J. Thiono, Stephan Kudlacek, John Forsberg, Cesar A. Lopez, Helen M. Lazear, Shaomin Tian, Brian Kuhlman, Aravinda M. de Silva

University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

Flaviviruses are a group of arthropod-borne viruses that represent a major public-health threat all over the globe. Several hundred million people are infected by DENVs, of which many develop dengue fever or dengue hemorrhagic fever. The same populations are at risk to other closely related flaviviruses, such as Zika virus (ZIKV). ZIKV infection can lead to neurological disorders and has been linked to severe birth defects.Results from flavivirus vaccine trials demonstrate the importance of the "quality" of a neutralizing response for developing durable protective immunity. Neutralizing Ab quality refers to the capacity to recognize serotype-specific targets of complex structure on the surface of the virion. Recent studies have established that quaternary epitopes and larger antigenic sites displayed on flavivirus E protein homodimers, but not monomers, are major targets of type-specific and cross-protective neutralizing Abs. The current leading DENV and ZIKV vaccine candidates in clinical testing are based on live or killed virus platforms, which have safety issues, especially in flavivirus seronegative populations and pregnant women. Flavivirus subunit vaccines, however, have shown poor performance in preclinical studies, most likely because the antigens tested do not display the critical quaternary structure epitopes. Here, we produced stable recombinant DENV and ZIKV E protein homodimers that are recognized by strongly neutralizing DENV and ZIKV specific monoclonal antibodies. In mice, the dimeric antigens stimulate strongly neutralizing antibodies that target epitopes that are similar to epitopes recognized by human antibodies following natural virus infection. The monomer antigen stimulates low levels of E-domain III targeting neutralizing antibodies. In a challenge model, only E dimer antigen stimulates protective antibodies, not the monomer. These results highlight the importance of mimicking the highly structured flavivirus surface when designing subunit vaccines.

503

SIGNALS OF ANTIGENIC DISTANCES EMBEDDED IN DENV PROTEINS - BEYOND THE SURFACE

Angkana T. Huang[1], Henrik Salje[2], Ana Coello Escoto[3], Nayeem Chowdhury[3], Christian Chávez[3], Bernardo Garcia-Carreras[3], Wiriya Rutvisuttinunt[4], Irina Maljkovic Berry[4], Chonticha Klungthong[1], Butsaya Thaisomboonsuk[1], Ananda Nisalak[1], Isabel Rodriguez-Barraquer[5], Damon W. Ellison[4], Anthony R. Jones[1], Stefan Fernandez[1], Timothy Endy[6], Derek J. Smith[2], Richard Jarman[4], Stephen S. Whitehead[7], Derek A. Cummings[3], Leah Katzelnick[8]

[1]*Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand,* [2]*University of Cambridge, Cambridge, United Kingdom,* [3]*University of Florida, Gainesville, FL, United States,* [4]*Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [5]*University of California, San Francisco, CA, United States,* [6]*State University of New York Upstate Medical University, Syracuse, NY, United States,* [7]*National Institutes of Health, Bethesda, MD, United States,* [8]*University of California, Berkeley, Berkeley, CA, United States*

The degree of accumulated immunity in the population against past circulating viral strains to emerging strains is important for understanding transmission dynamics. Changes in viral surface proteins affect the binding of antibodies and neutralization. However, little is known about how non-surface proteins affect antigenic features. We used a dataset of 286 dengue virus (DENV) strains that circulated in Bangkok, Thailand between 1994 to 2014 to test how amino acid changes across the full DENV genome relate to antigenic characteristics, measured as log2 plaque reduction neutralization test titers for twenty global reference DENV antisera. Effect sizes of amino acid substitutions in all sixteen DENV proteins were estimated with correlated substitutions grouped as clusters to avoid collinearity. Predictions of antigenic distances between DENV pairs were made by summing effects of substitutions separating them. As expected, 100-fold Monte Carlo cross-validation (10% as test) suggests strong predictive power (low median root mean squared error [RMSE]) when modeled with substitutions in the E protein (0.97, range: 0.87, 1.09), with 81 substitution clusters with non-zero effect sizes. Interestingly, nonstructural protein 1 (NS1), NS2A, and RNA-dependent RNA polymerase (NS5) had similar predictive capacity; RMSE of 0.96 (0.86, 1.06), 0.96 (0.87, 1.08), and 0.95 (0.86, 1.07), respectively. We investigate whether signals for these non-surface proteins were confounded by the phylogeny shared with E. We re-estimated the effects using concatenated sequences of those proteins and E and observed similar RMSE. However, the number of clusters with non-zero effect sizes declined from 77 to 34 (NS1), 76 to

40 (NS2A), and 75 to 44 (NS5). For clusters with effects retained, 21, 14, and 18 observed decline in effect sizes, respectively, while 9, 19 and 19 clusters showed increases. In addition, some clusters which had zero-effect when estimated with the single proteins had non-zero effects once adjusted for E. These mixed results hint at the possibility that non-surface proteins may contribute to antigenic differences.

504

ZIKA VIRUS INFECTION ENHANCES FUTURE RISK OF SEVERE DENGUE DISEASE

Leah Katzelnick[1], César Narvaez[2], Sonia Arguello[2], Brenda Lopez Mercado[2], Damaris Collado[2], Oscarlett Ampie[2], Douglas Elizondo[2], Tatiana Miranda[2], Fausto Bustos[1], Juan Carlos Mercado[3], Krista Latta[4], Amy Schiller[4], Bruno Segovia-Chumbez[5], Sergio Ojeda[2], Nery Sanchez[2], Miguel Plazaola[2], Josefina Coloma[1], M. Elizabeth Halloran[6], Lakshmanane Premkumar[5], Aubree Gordon[4], Federico Narvaez[2], Aravinda de Silva[5], Guillermina Kuan[7], 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]*Sustainable Sciences Institute, Managua, Nicaragua,* [3]*Laboratorio Nacional de Virología, Centro Nacional de Diagnóstico y Referencia, Ministry of Health, Managua, Nicaragua,* [4]*Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, MI, United States,* [5]*Department of Microbiology and Immunology, University of North Carolina School of Medicine, Chapel Hill, NC, United States,* [6]*Department of Biostatistics, University of Washington, Seattle, WA, United States,* [7]*Centro de Salud Sócrates Flores Vivas, Ministry of Health, Managua, Nicaragua*

Whether Zika virus (ZIKV) infection, like infection with a dengue virus (DENV1-4), increases future risk of dengue disease is unknown. We investigated prospective pediatric cohorts in Nicaragua that experienced sequential DENV1-3 (2004-15), Zika (2016-17), and DENV2 (2018-20) epidemics. We used relative risk regression (log-binomial models) to evaluate how prior DENV and ZIKV infection histories and antibody titers modify subsequent risk of symptomatic and severe DENV infections and symptomatic ZIKV infections (n=8399 total participants, 2-16 years old). Models were adjusted for age and sex. During the 2019-20 dengue epidemic (n=302 dengue cases, 3434 at risk), children with one prior ZIKV infection were at significantly elevated risk of symptomatic DENV2 infection (probability of 12.1%, 95% CI: 9.9-14.5) compared to flavivirus-naïve children (3.5%, 2.4-4.6), with comparable risk to children with one prior DENV infection (9.2%, 4.6-14.5). However, whereas children with >2 DENV infections (2.5%, 0.0-9.0) were not at greater risk of disease, children with one DENV infection followed by ZIKV infection remained at elevated disease risk (9.5%, 6.7-13.0). Importantly, prior ZIKV infection was also a significant risk factor for severe dengue disease. The probability of Dengue with Warning Signs/Severe Dengue and Dengue Hemorrhagic Fever/Dengue Shock Syndrome, both in the full cohort or only among DENV cases, was significantly increased for children with only prior DENV infection, prior ZIKV infection, or one DENV and one ZIKV infection. Further, intermediate anti-DENV antibody titers induced by previous ZIKV or DENV infection enhanced future risk of DENV2 disease and severity, both before the arrival of ZIKV (2004-2015) and after (2019-20). In contrast, in the pre-Zika era, high anti-DENV antibody titers protected against DENV1, DENV3, and ZIKV disease. Our findings point to fundamental differences in immunological interactions between ZIKV and specific DENV serotypes as well as among DENV1-4. That ZIKV infection can modulate dengue disease severity like a DENV serotype poses challenges to development of dengue and Zika vaccines.

505

SKIN-BASED PROTECTIVE IMMUNITY THROUGH REPEATED HOOKWORM INFECTION

Marie-Astrid Hoogerwerf, Vincent P. Kuiper, Jacqueline J. Janse, Roos van Schuijlenburg, Marijke C. Langenberg, Beckley A. Nosoh, Yvonne C. Kruize, Jeroen C. Sijtsma, Eric A. Brienen, Leo G. Visser, Lisette van Lieshout, Maria Yazdanbakhsh, Meta Roestenberg

Leiden University Medical Center, Leiden, Netherlands

Repeated exposure to hookworm L3 larvae in endemic areas does not lead to protective immunity, possibly caused by immunotolerance induced by the adult worms. If maturation of larvae is prevented through chemo- or radiation attenuation, repeated exposure can induce protective immunity in animal models. This protective immune response is thought to target the skin- or lung stage but has not been replicated in humans. We aimed to investigate protective efficacy of short-term exposure to infective larvae against subsequent hookworm challenge in humans. 23 healthy volunteers were randomized double-blind in a 2:1 ratio to three exposures to 50 *Necator Americanus* L3 at three week intervals, or placebo (repeated exposure phase). Each infection was abrogated after two weeks with albendazole. 12 weeks after first exposure all volunteers were challenged with two doses of 50 L3, followed for 16 weeks and then treated with albendazole (challenge phase). At each visit adverse events, eosinophils and fecal samples were collected. Primary endpoint was egg load, defined as the mean eggs per gram feces (epg) measured by Kato-Katz at 12-16 weeks after first challenge. 13 volunteers in the intervention group and 5 in the placebo group completed follow-up and were included in the per protocol analysis. During the repeated exposure phase of the study, no eggs were detected in volunteers' feces. Abdominal adverse events occurred but were mostly mild. Itching and skin rash were observed frequently and were severe in nine and five volunteers respectively in the intervention group. Both itching and skin rash increased in severity with subsequent exposures. In the challenge phase, egg load was lower in the intervention group than the placebo group (geomean 574 epg vs. 766 epg), although not statistically significant (p=0.24). However, egg loads were significantly lower in volunteers with severe rash as compared to those with mild or moderate rash (450 epg vs 704 epg, p=0.007). We conclude that repeated exposure to hookworm L3 larvae can induce protective immunity, likely targeting the skin stage. This finding offers exciting opportunities for hookworm vaccine development.

506

PROTEOMIC ANALYSIS OF *ASCARIS* LARVAE EXCRETORY-SECRETORY PRODUCTS

Grace Adeniyi-Ipadeola, Antrix Jain, Sung Yun Jung, Jill Weatherhead

Baylor College of Medicine, Houston, TX, United States

Ascariasis affects approximately 800 million people globally and causes significant morbidity. Upon oral infection with *Ascaris* eggs, the eggs hatch into L3 stage larvae and migrate from the intestines to the lungs via the circulatory system. While in the lungs, the L3 develop into L4 stage larvae. The L4 ascend the bronchotracheal tree and are subsequently swallowed into the intestines to develop into adult worms. Excretory-secretory (ES) product, released at each larval stage, is composed of proteins potentially important for *Ascaris* larval development, larval migration and induction or mitigation of the host immune response. The aim of this study was to identify ES proteins at different larval stages (L3-egg, L3-lung, and L4-trachea) that could be therapeutic targets to inhibit larval development and migration. L3-egg larvae were hatched and cultured *in vitro* in RPMI. L3-lung and L4-trachea were collected from infected mice *in vivo*, and subsequently cultured *in vitro*. A total of 40mL volume of ES product from each larval stage was used for proteomic analysis. Proteins were concentrated and digested using trypsin enzyme followed by peptide desalting and vacuum drying. Using LC-MS/MS, 270 unique proteins within larval ES product were identified. In total, 29 proteins were significantly enriched over 4-fold in L3-lung compared to L3-egg

and 26 proteins significantly enriched over 4-fold in L3-lung compared to L4-trachea (volcano plot). There was no difference between L3-egg and L4-trachea ES. Panther software analysis characterized enriched proteins in L3-lung as mostly glycolytic enzymes. Principal component analysis (PCA) noted relatedness between L4-trachea and L3-egg ES product while L3-lung clustered independently (PC1 55.53%, PC2 14.08%) suggesting larvae traversing lung have a distinct proteomic profile. Upregulated proteins unique to L3-lung may be important for larval nutrient acquisition through glycolysis and may fuel migratory and development phases of *Ascaris*. The functional role of *Ascaris* ES product makes it a potential target for future therapeutic development.

507

STRONGYLOIDES STERCORALIS CO-INFECTION MODULATES THE CEREBROSPINAL FLUID IMMUNE PROFILE IN TUBERCULOUS MENINGITIS

Joseph Donovan, Trinh Thi Bich Tram, Nguyen Hoan Phu, Nguyen Thi Thu Hiep, Le Van Tan, Nguyen Thuy Thuong Thuong, Guy E. Thwaites

Oxford University Clinical Research Unit, Ho Chi Minh City, Viet Nam

Tuberculous meningitis (TBM) is the most severe form of tuberculosis. Host neuroinflammatory responses are often excessive and dysregulated, contributing to poor outcomes. The soil transmitted helminth *Strongyloides stercoralis* is a neglected tropical disease endemic to Vietnam. A pro-inflammatory 'type 1' immune response of IFN-γ, TNF-α and IL-2 cytokines predominates in TBM. However, helminths induce a 'type 2' immune response, with eosinophils, IgE, and downregulation of type 1 immunity. In TBM *S. stercoralis* co-infection, contrasting immune responses may impair optimal immunity. In a pulmonary TB study, plasma IFN-γ, TNF-α, and IL-2 were reduced in TB *S. stercoralis* co-infection vs. TB alone. We hypothesised an immunomodulatory and potentially protective effect of *S. stercoralis* in TBM. Vietnamese adults with clinical TBM were enrolled in 2 randomised clinical trials of adjunctive dexamethasone therapy. Participants were screened for *S. stercoralis* by stool microscopy, stool PCR, and serology, and considered *S. stercoralis* 'positive' if one or more tests were positive, and 'negative' if all three tests were negative. Baseline cerebrospinal fluid (CSF) was centrifuged, and supernatant frozen at -80^{0}C prior to cytokine testing (IFN-γ, TNF-α, IL-2) by multiplex assay. Baseline data and clinical outcomes were recorded. Overall, 9.2% (63/686) participants tested positive for *S. stercoralis*. Median log2 CSF cytokine concentrations and clinical outcomes were compared between 58/63 positive cases and 105/110 negative controls. Median CSF concentrations of IFN-γ, TNF-α, and IL-2, were all significantly reduced in positive cases vs. controls (5.81 vs. 4.41 p=0.007; 3.58 vs. 2.51 p=0.014; 5.77 vs. 4.90 p=0.007, respectively). Reduced grade 3 disease and CSF WBC in the positive vs. control group supported a mechanism of reduced inflammation. Neurological complications by 3 months were reduced in the positive group, suggesting this immunomodulation may improve outcomes. *S.* stercoralis co-infection may modulate immune responses, reduce neuroinflammation, and improve clinical outcomes, in adults with TBM.

508

TAENIA SOLIUM-INDUCED AUTOANTIBODIES IN THE CEREBRAL SPINAL FLUID OF PATIENTS WITH SUBARACHNOID NEUROCYSTICERCOSIS

Aissatou Bah, Joshua Sciurba, Theodore E. Nash, Thomas Nutman

The National Institute of Health, Bethesda, MD, United States

It has been demonstrated that helminth infections can drive the production of autoantibodies that cause human disease through molecular mimicry. To identify potentially important autoantibodies in the pathogenesis of subarachnoid neurocysticercosis (SANCC), pooled cerebral spinal fluid (CSF) from 9 subjects with active SANCC and 9 healthy donors were tested for IgG that reacted to a human proteome microarray containing over 80% of the human proteome. The top 15 proteins on the array that were differentially reactive in those with SANCC were chosen to be expressed in RUC-containing constructs for further analyses using Luciferase Immunoprecipitation System (LIPS) assays. Serum samples from 24 individuals with SANCC showed markedly higher IgG reactivity in 7 of the 15 constructs tested compared with 14 healthy controls ($p < 0.05$). Furthermore, these 7 human proteins had *Taenia solium* homologues that were likewise expressed and tested for IgG sero-reactivity. Reactivity to 3 of these human proteins known to be expressed in the CNS (C1QL1, SMARCE1, and EXTL3) was highly correlated ($p < 0.05$) to the reactivity to their *T. solium* homologues (TsM_000389, TsM_0000474, TsM_0008236 respectively). These data suggest that among SANCC patients, *T. solium*-driven cross-reactive antibodies may have a role in mediating inflammation or pathology in SANCC. The functional implications of these findings are still underway. However, further elucidation of the targets and consequences of these potentially deleterious autoantibodies could enable mitigation of some pathology seen in NCC through the use of highly targeted therapeutics.

509

ALBENDAZOLE-INDUCED DAMAGE TO *TAENIA CRASSICEPS*CYSTS REQUIRES EOSINOPHILS FOR MAXIMAL EFFECT IN A MOUSE INTRAPERITONEAL MODEL

Joshua C. Sciurba, Pedro H. Gazzinelli-Guimarães, Theodore E. Nash, Thomas B. Nutman, Elise M. O'Connell

National Institutes of Health, Bethesda, MD, United States

Subarachnoid neurocysticercosis (SANCC) is characterized by a large cyst burden and a widely variable degree of inflammation and response to anthelmintic therapy. The mediators involved in controlling cyst growth and causing cyst death in SANCC are not fully understood, but eosinophils are commonly seen to a variable degree in the patient CSF. Converse to other helminth infections, studies using a *T. crassiceps* intraperitoneal mouse model have suggested that type 2 immune polarization (including M2 macrophages and eosinophils) may be favorable to cyst survival. To investigate the role of eosinophils in cyst death, we infected both BALB/c and eosinophil deficient (dblGATA) mice, intraperitoneally with 20 non-budding *T. crassiceps* cysts (<2mm in diameter). At day 28 post infection, we found that the cyst volume and viability of dblGATA and BALB/c animals were not significantly different. To address the role of eosinophil dependent cyst killing following anthelmintic therapy, the same model was used but albendazole (100 µL of 10 µg/mL solution) was administered orally to the mice for 3consecutive days prior to the experiment endpoint on day 28. As expected, the infected BALB/c mice treated with albendazole had reduced cyst burden compared to infected BALB/c mice not treated with albendazole (cyst volume: 8mL vs 11mL, $p < 0.05$) and decreased cyst viability by water soluble tetazolium (WST-1) assay (WST signal to noise: 3.4 vs 4.2, $p < 0.05$). However, inthe dblGATA mice albendazole had minimal effect on cyst burden. Indeed, in comparing the BALB/c to the dblGATA following albendazole administration, the dblGATA mice had significantly greater cyst burden compared to BALB/c animals (cyst volume: 14mL vs 8mL, $p < 0.05$) as well as increased cyst viability (WST signal to noise: 4.3 vs 3.4, $p < 0.05$). Together, these findings suggest that in the mouse model, eosinophils appear to play an important role inmediating cyst death in the context of anthelmintic therapy.

510

HISTOLOGICAL FINDINGS IN THE CALCIFIED LESIONS IN NEUROCYSTICERCOSIS DISEASE IN A STUDY LONGITUDINAL IN PIGS

Laura E. Baquedano Santana, Javier Bustos, Noemi Miranda, Jaime Caceres, Hector Garcia

Universidad Peruana Cayetano Heredia, Lima, Peru

Neurocysticercosis (NCC) is a parasitic disease caused by the Taenia solium larval stage (cyst) in the central nervous system. It is the leading cause of acquired epilepsy in endemic countries. Calcifications produced

by this disease are associated with seizures in 20% of patients. This study aimed to characterize and evaluate the neuropathological findings of calcifications in the brain of pigs with NCC and treated with bisphosphonates after calcification of the parasite. We used 14 pigs naturally infected with T. solium, and they received antiparasitic treatment. Then they were treated with bisphosphonates (group experimental) and another group was not treated (control group). Were sacrificed at eight months post-treatment and the brains were perfused, removed, and fixed in paraformaldehyde. Hematoxylin-Eosin and Masson's Trichrome stain were used to describe the response inflammatory. Also, we use Von Kossa and Alizarin red stain to identify and describe the presence of calcium deposits. Immunohistochemistry studies were evaluated with IBA, GFAP, vWF, and NF. The pigs with NCC treated with the drug had an inflammatory response significant after treatment. We found differences in collagen scarring, microglial reactivity, calcium accumulation, and axonal damage in the classic morphological appearance of traumatic axonal injury. Our data provide novel insight into the relationship between axonal damage, disrupted blood-brain barrier (BBB), glial reactivity, and the progression of calcification of the neurocysticercosis and new drugs on the research of antiparasitic treatments.

511

ESTABLISHING THE PRESENCE AND IMPACT OF PORCINE CYSTICERCOSIS IN HISPANIOLA

Yussaira Castillo Fortuna, Arve Lee Willingham

Ross University School of Veterinary Medicine, Basseterre, Saint Kitts and Nevis

Taenia solium taeniosis /cysticercosis, transmitted between people and pigs, has been ranked the most important foodborne parasitic hazard globally and a common cause of preventable epilepsy within endemic areas. Despite being known as the 'pork tapeworm', all individuals, including people who do not eat pork for ethical, religious or cultural reasons, are at risk of developing cysticercosis transmitted from a human tapeworm carrier. This zoonotic parasite, *T. solium*, is considered endemic across Latin America. Yet, little information is available for Central America and the Caribbean basin, indicating that *T. solium* infections are likely underreported. In Hispaniola, cysticercosis is considered nonexistent among a population that attribute the symptoms of epilepsy caused by the parasite to voodoo and sorcery. We conducted a study to determine the presence and prevalence of porcine cysticercosis as a sentinel of transmission in rural communities across Hispaniola. Elias Piña and San Juan provinces, both located in the most impoverished region of the Dominican Republic, an area bordering Haiti where 30% of human epileptics were found positive for neurocysticercosis, were selected for the study. All pigs kept in extensive production systems were considered with 154 pigs of both sexes and different age ranges (over 3 months) within households in these provinces randomly selected for the survey. Pigs were examined for cysticercosis using the lingual examination method and an antigen enzyme-linked immunosorbent assay (Ag-ELISA apDia). In Elias Piña, 34.7% of the sampled animals were positive for cysticercosis, while 21.5% tested positive in San Juan Province. The most significant risk factors associated with cysticercosis prevalence in this region were free-roaming of pigs across the border with Haiti, sourcing of water from rivers around the communities and the lack of knowledge of the population regarding the disease. Due to the lack of movement control of free-roaming pigs across the border and tendency of the human population to disregard the border, we have initiated a study of the situation "cross border" into Haiti.

512

DIFFERENTIAL GENE EXPRESSION OF HUMAN NEUTROPHILS FROM SUBCLINICAL AND CLINICAL *LEISHMANIA BRAZILIENSIS* INFECTION

Jacilara Alexandrino Conceicao[1], Ednaldo Lago[2], Pedro Carneiro[2], Andreza Dorea[2], Walker Nonato[2], Aline Muniz[2], Eric Aguiar[3], Diogo Valadares[1], Edgar Carvalho[4], Maria Olivia Bacellar[2], Mary Wilson[1]

[1]*University of Iowa, Iowa City, IA, United States,* [2]*Federal University of Bahia, Salvador, Brazil,* [3]*State University of Santa Cruz, Ilheus, Brazil,* [4]*Goncalo Moniz Research Center, Salvador, Brazil*

Individuals with *Leishmania braziliensis* subclinical (SC) infection are characterized by a positive leishmania skin test and/or high production of IFN-γ specific to leishmania antigen and the absence of clinical disease manifestations. Our preliminary data showed that SC neutrophils present phenotypical differences and higher ability to control *in vitro* infection with *L. braziliensis* than neutrophils from patients with cutaneous leishmaniasis (CL). We hypothesize that this profile are due transcriptomic differences in neutrophils from both groups. To investigate differential gene expression that may be related to protection or susceptibility to the infection, a transcriptional study was performed using anti-CD15 ultra-purified neutrophils obtained from peripheral blood from SC individuals, CL patients and health controls. RNA-Seq analysis show upregulation of CASP1 (fold change= 2.24, P<0.001), BTN3A1 (fold change= 2.26, P<0.001) and BTN3A3 (fold change= 2.47, P<0.001) in CL neutrophils when compared to SC neutrophils. CASP1 plays a central role and inflammasome activation and is associated to severity of lesions and CD8+ T cells cytotoxicity observed in CL. Although BTN3A1 and BTN3A3, proteins homologous to B7, are increased in PMN from CL patients, additional experiments will be performed to investigate protein surface expression and ability of those cells to modulate the TCR-induced T cells activation. In conclusion, in contrast to neutrophils from CL patients, neutrophils from SC present lower expression of transcripts associated to tissue damage, pointing out neutrophils plasticity during *L. braziliensis* infection. Ongoing studies will validate the data obtained by RNA-Seq assessing the functional implications of this transcript expression during *L. braziliensis* infection and the possibility of using them as biomarkers for CL.

513

MTOR MEDIATED IMMUNE CELL MIGRATION LEADS TO IMMUNOPATHOLOGY DURING *LEISHMANIA MAJOR* INFECTION

Gopinath Venugopal[1], Jordan Bird[2], Hayden Roys[1], Charity Washam[2], Anne Bowlin[1], Manjunath Bettadapura[3], Stephanie Byrum[2], Tiffany Weinkopff[1]

[1]*Dept. of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, AR, United States,* [2]*Dept. of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, AR, United States,* [3]*Dept. of Biology, University of Arkansas at Little Rock, Little Rock, AR, United States*

Leishmania species are the causative agents of cutaneous leishmaniasis, a parasitic disease characterized by the presence of skin lesions. During infection both the parasites and the inflammatory infiltrate contribute to disease. Bulk transcriptomic RNASeq analysis revealed pathways involved in leukocyte trans-endothelial migration, cell adhesion, and chemokine signaling were enhanced in leishmaniasis. In general, immune cell migration is mediated by blood endothelial cells (BECs) binding immune cells and guiding them across the endothelium into the inflamed tissue. However, the mechanisms by which BECs mediate cellular entry into dermal lesions during *Leishmania* infection is poorly understood. Given immunopathology contributes to disease severity, we sought to investigate the molecular mechanisms responsible for immune cell migration into the tissue. scRNASeq analyses between naïve and *L. major*-infected mice revealed cellular heterogeneity including distinct resident and recruited

cell types in the skin following murine *L. major* infection. We found BECs from infected skin express elevated transcripts for selectins and adhesion molecules, while concomitantly downregulating transcripts responsible for junctional stability. During infection BECs sense hypoxic conditions in the tissue which is associated with mTOR activation. mTOR target gene expression derived from transcriptomic data reflects mTOR activation in BECs that could possibly support immune cell migration into the dermal lesions. To determine if mTOR signaling contributed to BEC activation, mice were treated with rapamycin, an mTOR inhibitor. Rapamycin treatment decreased BEC selectins and adhesion molecules such as VCAM-1 which reduced the inflammatory infiltrate leading to smaller lesions following *L. major* infection. Altogether, this comprehensive dataset shows immune cell entry into the dermal lesions is mediated by BEC mTOR signaling during leishmaniasis.

515

BLOOD FEEDING AND SALIVA OF DISEASE VECTORS TRIGGER HEME OXYGENASE-1 PRODUCTION IN HOST SKIN

Thiago Soares de Souza Vieira[1], Eva Iniguez[1], Tiago Donatelli Serafim[1], Waldione de Castro[1], Subir Karmakar[2], Maria Disotuar[1], Pedro Amado Cecilio[1], Joshua Lacsina[1], Claudio Meneses[1], Bianca Nagata[1], Silvia Cardoso[3], Daniel Sonenshine[1], Ian Moore[1], Valeria Borges[4], Ranadhir Dey[2], Miguel Soares[3], Hira Nakhasi[2], Fabiano Oliveira[1], Jesus Valenzuela[1], Shaden Kamhawi[1]

[1]*NIH NIAID, Rockville, MD, United States,* [2]*Food and Drug Administration, Silver Spring, MD, United States,* [3]*Instituto Gulbenkian de Ciência, Oeiras, Portugal,* [4]*Instituto Goncalo Moniz - Fiocruz Bahia, Salvador, Brazil*

Arthropod vectors are responsible for transmission of several deadly or debilitating pathogens that cause malaria, visceral leishmaniasis, Zika, Dengue, and Lyme disease, among many others. Blood-feeding by arthropod vectors leads to inflammation and leakage of red blood cells (RBCs) in host skin. This will possibly culminate in the release of heme from RBCs, which promotes tissue damage. Heme oxygenase-1 (HO-1) is a cytoprotective enzyme that catabolizes heme to prevent heme-mediated cell death. Its enzymatic activity has broad cytoprotective and immunoregulatory effects targeting a plethora of genes involved in stress responses. A growing body of evidence has demonstrated that HO-1 affords protection against diseases such as hepatitis B, tuberculosis, sepsis and malaria. Previously, we have shown that HO-1 is induced by resident macrophages, and to a lesser extent by inflammatory macrophages, after sand fly bites. Induction of HO-1 is dependent on the transcriptional factor Nrf2 and is independent of IL-10. Importantly, HO-1 is also induced in human skin after exposure to bites of sand flies. Here, we extended our observations to show that HO-1 is a universal host response to bites of hematophagous arthropods including mosquitoes and ticks. *In vivo* production of HO-1 was associated with massive leakage of RBCs and increased levels of hemoglobin in the tissue. Intradermal injection of saliva from *Lutzomyia longipalpis* and *Aedes aegypti* also promoted release of hemoglobin and induction of HO-1, potentially due to the potent anti-hemostatic capability of vector saliva. Mechanical damage induced by a needle did not reproduce these effects. FACS analysis and confocal imaging identified skin-residing HO-1^{+}-iron recycling macrophages that erythrophagocytose RBCs in injured skin after insect bites. We are currently molecularly phenotyping the various macrophage subpopulations associated to HO-1 production by RNA sequencing. Collectively, our data demonstrate that HO-1 induction through erythrophagocytosis is a universal host response to capillary laceration and saliva deposition by blood-feeding arthropods.

516

PERTURBATION OF CD4 T CELL RESPONSE AFTER COMORBID TICK-BORNE INFECTION AND PROGRESSION OF CANINE LEISHMANIASIS

Breanna M. Scorza, Kurayi Mahachi, Erin Cox, Tyler Baccam, Shelbe Vollmer, Danielle Pessoa, Eric Kontowicz, Ogechukwu Chigbo, Christine A. Petersen

University of Iowa, Iowa City, IA, United States

Zoonotic Visceral Leishmaniasis in canines (CanL) is driven by transmission of protozoan *Leishmania infantum (Li)* parasites from canine reservoirs to humans. Identifying factors driving development of severe CanL is crucial to limiting transmission and detecting novel human immune response targets. IFNγ-secreting CD4+ T cells are critical to control intracellular *Leishmania* replication and CanL progression. Although dogs can remain asymptomatic for years, we have shown immune exhaustion occurs during symptomatic CanL, with reduced CD4+ T cell proliferation and IFNγ secretion in response to *Leishmania* antigen (Esch 2013). The factors controlling this immune switch remain unclear.Our group recently identified a significant association between tick-borne pathogen co-exposure (TBC) and CanL progression (Toepp 2019). However, the immune consequences of TBC relevant to CanL progression remain to be evaluated. We hypothesize TBC causes systemic inflammation in *Li*-infected asymptomatic hounds, leading to development of T cell exhaustion and symptomatic CanL.To prospectively measure the impact of TBC on CanL progression, 50 TBC seronegative dogs with subclinical *Li* were randomized into blinded groups receiving oral isoxazoline tick prevention, or placebo, from 2019-2020 across two tick transmission seasons. Using peripheral blood, T cell proliferation, inhibitory receptor expression, and cytokine production in response to *Li* antigen and TBC were assayed at three-month intervals. Physical examination, complete blood count, and chemistry panels were used to evaluate CanL severity according to LeishVet staging guidelines. Combining these measurements, the association between TBC with development of immune exhaustion over time was compared in 4Dx SNAP seronegative vs. seropositive dogs. This is the first demonstration that tick-borne co-infection alters the inflammatory profile of T cells present in asymptomatic *Li* infection prior to progression to clinical CanL. Further, it tests the efficacy of prevention of tick infestation and subsequent disease prevents progression of CanL as a potential public health intervention.

517

IMMUNIZATION WITH SECOND GENERATION LEISHMANIA VACCINE, *L. MAJOR* CENTRIN GENE DELETED PARASITES, INDUCES SKIN RESIDENT MEMORY T CELLS THAT PLAY A ROLE IN PROTECTION AGAINST INFECTION

Nevien Ismail, Subir Karmakar, Parna Bhattacharya, Kazuyo Takeda, Ranadhir Dey, Hira L. Nakhasi

US FDA, Silver Spring, MD, United States

Leishmaniasis is a vector-born disease transmitted through sand fly bite with no available vaccine. First generation vaccination through leishmanization with wild type *Leishmania major* (*LmWT*) has been used successfully but is not safe. Recently, we have demonstrated that immunization with second generation vaccine *Leishmania major* centrin gene-deleted parasites (*LmCen*$^{-/-}$) protects against infection via induction of host cellular immunity. Resident memory T cells (TRMs) are considered the first line of defense against infections invading the host through epithelial or skin barrier. We evaluated and compared the generation and function of skin TRMs post *LmCen*$^{-/-}$ immunization, with *LmWT* infection i.e. leishmanization in mice. At 15 weeks post immunization, the skin of immunized mice had significantly higher population of TRMs compared to leishmanized mice. Concurrently, the expression of chemokine receptors CCR8, CXCR6 and CXCR3 and cytokines that control generation and survival of skin TRMs was significantly higher in the skin of immunized mice, compared to leishmanized mice. Next, we measured the recruitment and effector function of TRMs after infection. Upon virulent challenge

with *LmWT* (3 days post challenge), immunofluorescence labeling of skin sections, showed rapid recruitment of TRMs in both the immunized and leishmanized mice. In addition, both immunized and leishmanized mice recruited significantly higher circulating T cells to the site of infection post challenge compare to non-immunized mice. IFNγ expression by both CD4 and CD8 skin TRMs post challenge was significantly higher in immunized and leishmanized mice compared to non-immunized mice. Interestingly, we observed significantly higher expression of Granzyme-B in skin CD4 TRMs of immunized mice compared to both leishmanized and non-immunized mice. Taken together, these results show that immunization with second generation vaccine (*LmCen*$^{-/-}$) generates functional population of skin TRMs which play an important role in protection against *Leishmania* infection.

518

GLP GRADE *LEISHMANIA MAJOR* (*LMCEN-/-*) INDUCES A ROBUST HOST PROTECTIVE IMMUNE RESPONSE AGAINST VECTOR BITE TRANSMITTED VISCERAL LEISHMANIASIS IN PRECLINICAL ANIMAL MODEL AS WELL AS IMMUNE RESPONSE IN HUMAN PBMCS FROM ENDEMIC AREA

Subir Karmakar[1], Nevien Ismail[1], Fabiano Oliveira[2], James Oritian[2], Wen Wei Zhang[3], Kamaleshwar Singh[4], Swarnendu Kaviraj[4], Sushmita Das[5], Tiago Serafirm[2], Monica Satoskar[1], Sanika Satoskar[1], Raj Sastry[1], Shinjiro Hamano[6], Abhay Satoskar[7], Greg Matlashewski[8], Shaden amhawi[2], Pradeep Das[5], Sanjay Singh[9], Ranadhir Dey[1], Jesus G. Valenzuela[2], Hira L. Nakhasi[1]

[1]*US FDA, Silver Spring, MD, United States,* [2]*NIAID/NIH, Rockville, MD, United States,* [3]*McGill University, Montreal, QC, Canada,* [4]*Gennova Biopharmaceuticals, Hinjawadi Phase II, India,* [5]*RMRIMS,, Patna, India,* [6]*Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan,* [7]*Department of Pathology and Microbiology, Ohio State University, Columbus, OH, United States,* [8]*Department of Microbiology and Immunology, McGill University, Montreal, QC, Canada,* [9]*Gennova Biopharmaceuticals, Hinjawadi Phase II, Pune,, India*

Visceral Leishmaniasis (VL) is a vector-borne parasitic disease and is fatal if non-treated. To date there is no licensed vaccine available against human leishmaniasis. Low dose of dermatotrophic *Leishmania major* infection confers protection against cutaneous leishmaniasis (CL) called leishmanization. However, such a method of immunization is not practical because of the greater risk of infection in a naïve population. Therefore, genetically modified live attenuated parasites that are non-pathogenic might induce similar protective immunity as leishmanization. We have developed centrin-gene deficient *Leishmania major (LmCen-/-)* using CRISPR-Cas methodology and evaluated the safety, immunogenicity as well as cross-protective efficacy against *L. donovani* challenge in hamster model. Previously, we have demonstrated *LmCen-/-*, grown under laboratory conditions, induced a strong pro-inflammatory immune response and protected immunized hamsters against *L. donovani* infection transmitted by sand fly bites. To evaluate the vaccine safety and efficacy in future human studies, we developed GLP grade (cGMP compliant) *LmCen-/-*. We demonstrated that GLP grade *LmCen-/-* parasite induced pro-inflammatory immune response and host protection against sand fly mediated *L. donovani* infection comparable to lab grown *LmCen-/-*. More importantly, we also have demonstrated induction of cellular immune response in the PBMCs isolated from healed VL as well as people living in VL endemic region. Our studies demonstrate that the *LmCen-/-* mutant parasite is safe as an immunogen and has a potential to be an effective vaccine against VL and can proceed to be tested in humans.

519

COMMUNITY-BASED GUINEA WORM SURVEILLANCE IN CHAD: EVALUATING A SYSTEM AT THE INTERSECTION OF HUMAN AND ANIMAL DISEASE

Beth L. Rubenstein[1], Sharon Roy[1], Karmen Unterwegner[2], Sarah Yerian[2], Adam Weiss[2], Hubert Zirimwabagabo[3], Elisabeth Chop[3], Mario Romero[3], Philip Tchindebet[4], Tchonfienet Moundai[4], Sarah Guagliardo[1]

[1]*Centers for Disease Control and Prevention (CDC), Atlanta, GA, United States,* [2]*The Carter Center, Atlanta, GA, United States,* [3]*The Carter Center, N'Djamena, Chad,* [4]*Ministry of Public Health, N'Djamena, Chad*

Guinea worm disease is a debilitating parasitic infection. In 1986, The Carter Center and partners launched an international program for Guinea worm eradication. Since then, annual human Guinea worm cases have dropped from approximately 3,500,000 across 20 countries in 1986 to 54 across 3 countries in 2019. However, recent identification of canine cases in Chad threatens this progress. We aimed to assess the effectiveness of community-based Guinea worm surveillance in Chad, with special attention to the detection and containment of canine cases.We performed a mixed-methods evaluation. We administered a quantitative survey measuring system fidelity and inputs among 627 respondents (villagers, local leaders, community volunteers and supervisors) across 45 villages under active surveillance. Another qualitative evaluation examined system usability and outputs based on key informant interviews with 11 Guinea worm program staff. We defined containment as methods preventing dogs from contaminating water sources. Villagers visited by a community volunteer at least twice per week had better knowledge of Guinea worm symptoms (odds ratio [OR]: 1.71; 95% confidence interval [CI]: 1.04-2.79) and could name more strategies for prevention (OR: 2.04; 95% CI: 1.32-3.15), compared with villagers visited less frequently. Knowledge gaps about reasons for reporting Guinea worm and the importance of canine containment existed across all respondent categories. Interviews identified several informatics processes that were inefficient or redundant, and exposed concerns specific to canine surveillance about sensitivity and containment data quality. Community volunteers are central to Guinea worm surveillance in Chad, but many do not understand the public health rationale behind their work or the relevance of canine cases to human disease. To improve surveillance, we recommend retraining volunteers and their supervisors about why reporting matters, streamlining data collection processes, introducing canine case sweeps to more systematically search dogs for worms, and using photographs to validate correct canine containment.

520

PREVALENCE OF CRIMEAN-CONGO HEMORRHAGIC FEVER AMONG LIVESTOCK AND TICKS IN ZHAMBYL, KAZAKHSTAN

Lilit Kazazian[1], Yekaterina Bumburidi[2], Jonathan Bryant-Genevier[1], Victoria Seffren[1], Jennifer R. Head[1], Dmitriy Berezovskiy[2], Bakhytkul Zhakipbayeva[2], Stephanie J. Salyer[1], Barbara Knust[1], Cheng-Feng Chiang[1], Gulfaira Mirzabekova[3], Kumisbek Rakhimov[4], Jandar Koekeev[5], Kanatbek Kartabayev[6], Seydigapbar Mamadaliyev[7], Curtis Blanton[1], Trevor Shoemaker[1], Daniel Singer[2], Daphne B. Moffett[2]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Centers for Disease Control and Prevention – Central Asia Office, Almaty, Kazakhstan,* [3]*Zhambyl Oblast Department of Health, MoH, Taraz, Kazakhstan,* [4]*Zhambyl Oblast Department for Quality Control and Safety of Goods and Services, MoH, Taraz, Kazakhstan,* [5]*Zhambyl Oblast Veterinary Inspection, MoA, Taraz, Kazakhstan,* [6]*Zhambyl Oblast Veterinary Department of Oblast Administration, Taraz, Kazakhstan,* [7]*Central Reference Laboratory, Branch of NRCV, MoES, Almaty, Kazakhstan*

Crimean-Congo hemorrhagic fever (CCHF) is a highly fatal zoonotic disease endemic to Kazakhstan, commonly transmitted to humans from livestock via infected ticks. We previously identified risk factors and estimated a 1.2% human seroprevalence of CCHF among livestock-

owning households in the Zhambyl region of southern Kazakhstan. Recent laboratory results from livestock and ticks allowed us to further explore the human-animal interface of CCHF in Zhambyl. Thirty rural villages - 15 with a known history of CCHF circulation (endemic) and 15 without known circulation (non-endemic) - were selected by cluster sampling with probability proportional to livestock population size. Whole blood samples were collected from 521 sheep and 454 cattle in randomly selected households within each village. Any ticks found on the animals were collected for laboratory testing. Livestock blood was analyzed by enzyme-linked immunosorbent assay (ELISA) for evidence of past infection (CCHF-specific IgG antibodies). Samples from 234 ticks found on sheep and 236 ticks found on cattle were analyzed by reverse transcriptase real-time PCR to detect CCHF RNA and antigen-capture ELISA to detect CCHF antigen. Overall weighted seroprevalence was 5.74% (95% CI: 3.13, 10.32) among sheep and 22.54% (95% CI: 15.77, 31.16) among cattle. Weighted sheep seroprevalence was significantly higher in endemic (15.53%, 95% CI: 6.89, 31.68) compared to non-endemic villages (2.78%, 95% CI: 1.18, 6.42; $p<0.001$), whereas weighted cattle seroprevalence was not associated with known endemicity (endemic villages: 25.88%, 95% CI: 17.21, 36.98; non-endemic villages: 20.08%, 95% CI: 10.90, 34.02; $p=0.42$). Two CCHF-positive ticks were found on sheep (2.37%, 95% CI: 0.56, 9.46), and three CCHF-positive ticks were found on cattle (3.83%, 95% CI: 1.21, 11.48). Study findings underscore the need for public health measures to address the risk of CCHF even in areas without a known history of circulation.

521

ANIMAL OWNERSHIP AND INFANT FEEDING PRACTICES AS PREDICTORS OF CAMPYLOBACTER INFECTIONS IN INFANTS IN SHURUGWI DISTRICT, ZIMBABWE

C. Batsirai Mutasa[1], Robert Ntozini[1], Kuda Mutasa[1], Thompson Runodamoto[1], Marya P. Carmolli[2], Dzivaidzo Chidhanguro[1], Naume Tavengwa[1], Dorothy M. Dickson[2], Beth D. Kirkpatrick[2], Andrew Prendergast[1], E. Ross Colgate[2]

[1]*Zvitambo Institute for Maternal and Child Health Research, Harare, Zimbabwe,* [2]*University of Vermont Larner College of Medicine, Burlington, VT, United States*

Campylobacter spp are a major contributor to diarrheal disease in infants in low- and middle-income countries; however reservoirs and transmission pathways of Campylobacter remain poorly understood. Early infant feeding practices and cohabitation with animals may facilitate acquisition of enteric pathogens, including Campylobacter. This study was conducted to identify potential targets for interventions to prevent Campylobacter infections in children less than 12 months of age in Shurugwi District, Zimbabwe. We conducted a longitudinal study in an urban area where a total of 85 eligible mother-baby pairs were enrolled at a health facility or in their homes within 10 days of birth and followed monthly up to one year. At each monthly visit, infant stool samples were collected and cultured for Campylobacter on Modified Charcoal-Cefoperazone-Deoxycholate Agar (MCCDA) plates with catalase and oxidase confirmatory testing. A questionnaire on infant feeding practices and co-habitation with animals was administered to mothers by trained research nurses. To determine predictors of Campylobacter positive stools for inclusion in multivariable logistic regression analysis, we selected factors based on the literature and prevalence in our sample. Six predictors were identified: feeding the infant oral *muti* (traditional medicine), water or cooking oil; and the presence of chickens, dogs, or rats in or near the home. Campylobacter prevalence among 76 infants at baseline was 25% (N=19) and 13% (N=40) across 307 monthly follow up visits. Feeding children oral *muti*, water or cooking oil were not associated with Campylobacter infection, nor were ownership of chickens or dogs and the presence of rats (all odds ratios crossed one). Using a sandwich variance estimator to account for correlation within children with repeat positive samples and adjusting for infant age had no effect on the final model. Infant feeding practices and ownership of animals did not predict Campylobacter infection in young children. Further tests on banked environmental and maternal samples are required to understand the source of Campylobacter infections in this population.

522

MULTI-HOST, MULTI-PARASITE SCHISTOSOMIASIS IN AFRICA: A ONE HEALTH PERSPECTIVE IN OUR CHANGING WORLD

Elsa Leger[1], Anna Borlase[2], Cheikh B. Fall[3], Nicolas D. Diouf[4], Stefano Catalano[1], Aidan M. Emery[5], Momar Ndao[6], Babacar Faye[3], David Rollinson[5], James W. Rudge[7], Mariama Sene[4], Joanne P. Webster[1]

[1]*Royal Veterinary College, London, United Kingdom,* [2]*University of Oxford, Oxford, United Kingdom,* [3]*Universite Cheikh Anta Diop, Dakar, Senegal,* [4]*Universite Gaston Berger, Saint-Louis, Senegal,* [5]*Natural History Museum, London, United Kingdom,* [6]*McGill University, Montreal, QC, Canada,* [7]*London School of Hygiene & Tropical Medicine, London, United Kingdom*

Schistosomiasis is a Neglected Tropical Disease caused by *Schistosoma* parasitic worms. It inflicts a significant burden on human and animal populations, particularly across sub-Saharan Africa (SSA). While efforts to eliminate schistosomiasis are gathering momentum, the potential zoonotic risk posed by livestock *Schistosoma* species via viable hybridisation, and the implications of the multi-host aspects of schistosomiasis transmission for disease control in SSA, have been largely overlooked. The aim of our study was to quantify the disease burden in both human and livestock populations and the connection between the two in order to understand the multi-host, multi-parasite transmission cycle of schistosomiasis in Africa, and the implications for Global Health. Over three years, systematic randomized surveys of human and livestock definitive hosts, and snail intermediate hosts, were performed in two areas of Senegal, West Africa. We combined epidemiological, parasitological, and molecular analyses to elucidate the occurrence and distribution of *Schistosoma* species and hybrids. The prevalence of schistosomiasis was extremely high in human (up to 88% for urogenital schistosomiasis). High prevalence levels of schistosomiasis in livestock were also detected (up to 94% for *S. bovis* in cattle and repeated finding of livestock schistosomes hybrids between *S. bovis* and *S. curassoni*), representing a continued potential risk to human health via zoonotic transmission and hybridisation between livestock and human schistosomes. Viable hybrids between *S. haematobium* with *S. bovis* occurred frequently in humans and snails intermediate hosts, although none were not found in livestock. We highlight the implications for both human and animal health of the ongoing burden of schistosomiasis in Senegal, and the risks posed by zoonotic transmission. We demonstrate the impact that introgressive hybridisation, evolving host ranges and wider ecological conditions may have on the transmission dynamics of not only schistosomiasis, but other infective agents, and the need to consider control and elimination targets within a One Health framework.

523

CHICKFLOWS: A FOOD SYSTEMS APPROACH UTILIZING MICROBIAL MEASURES TO ASSESS KEY HAZARDS AND RISKS TO CHILD HEALTH ASSOCIATED WITH CHICKEN-RELATED ENTEROPATHOGENS IN MAPUTO, MOZAMBIQUE

Frederica Lamar[1], Amélia Mondlane-Milisse[2], Courtney Victor[1], Kelsey Jesser[1], Hermógenes Mucache[2], Matthew C. Freeman[1], Karen Levy[1]

[1]*Emory University, Atlanta, GA, United States,* [2]*Eduardo Mondlane University, Maputo, Mozambique*

The morbidity and mortality associated with exposure to animal feces is unquantified yet likely substantial in contributing to the global burden of diarrheal disease in children. Given small-scale poultry farming is being encouraged as a development strategy in low and middle-income countries, a value chain approach is necessary to understand risks of childhood exposure to chicken fecal contamination, in particular for *Campylobacter* and non-typhoidal *Salmonella*, which are common in poultry products. From July - September 2019, 118 pooled feces and 75 carcass rinse samples were collected from broilers, layers, and indigenous chickens at key settings along the chicken value chain: depots (stores selling day- old chicks), small-scale farms, informal markets, grocery stores,

and households. qPCR analyses were performed for *Salmonella* spp., *Campylobacter* spp., *C. jejuni*., and *Cryptosporidium* spp. Observations were conducted at informal markets, and semi-structured surveys were administered concurrently with sample collection. Contamination was detected across all settings. Prevalence of *Campylobacter* spp. was 81.8% (*N*=110) in feces and 88.7% (*N*=62) in carcass rinses, while prevalence of *Salmonella* spp. was 11.0% (*N*=118) in feces and 21.6% (*N*=74) in carcass rinses. Of samples positive for *Campylobacter* spp., 86.7% (*N*=60) of carcass rinses and 88.8% (*N*=89) of pooled fecal samples were positive for *C. jejuni*. Most notably, 96.3% (*N*=108) of informal market samples were positive for *Campylobacter* spp. *Cryptosporidium* spp. was not detected in any samples. Given the role poultry plays in foodborne disease transmission, mapping value chains for poultry production concurrent with assessing microbial contamination at key nodes is an essential link connecting the agricultural environment to pathogen transmission and subsequent food safety risks. Future studies should go beyond traditional water, sanitation, and hygiene exposure routes most commonly considered in the field of diarrheal disease research, to explore additional exposure routes and cross-contamination along food production systems.

524

LIVESTOCK AND THE EPIDEMIOLOGY OF SLEEPING SICKNESS: MECHANISMS AND IMPLICATIONS

Julianne Meisner, Jonathan Wakefield, David M. Pigott, Ali Rowhani-Rahbar, Jonathan D. Mayer, Peter M. Rabinowitz

University of Washington, Seattle, WA, United States

Human African trypanosomiasis (HAT) control efforts have reduced incident infections, however animal reservoirs threaten elimination goals. For the human-predominant gambiense form (gHAT) the role of animal reservoirs is uncertain, and elimination of the zoonotic rhodesiense form (rHAT) is considered impossible due to wildlife reservoirs. Our research seeks to close this gHAT knowledge gap and inform the feasibility of rHAT eradication with control of domestic animal reservoirs alone. We will present preliminary results from Malawi (rHAT) and South Sudan (gHAT). We created national maps of livestock (cattle and pig) density (livestock:human ratio) using survey and census data. Next, we used WHO Atlas of HAT data to estimate the effect of livestock density on HAT risk, accounting for dependence in space using the stochastic partial differential equations approach and adjusting for wealth, index events, and environmental variables. In Malawi, we stratified on time as treatment-confounder feedback precludes confounder adjustment in longitudinal models. In Malawi we have generated continuous (0.017° grid) annual maps over 2000-2016; median density was 0.07 (IQR 0.05, 0.10) for cattle and 0.10 (IQR 0.06, 0.14) for pigs, and 494 rHAT cases were reported. The rate ratio (RR) for a 50% increase in cattle density was 1.03 in 2005 (95% CI 0.99, 1.07), 1.05 in 2010 (95% CI 1.02, 1.08), and 1.08 in 2014 (95% CI 0.36, 6.36). For pigs the respective RR s were 1.08 (95% CI 1.03, 1.12), 1.09 (95% CI 1.05, 1.13), and 1.07 (95% CI 0.25, 4.44). In South Sudan, due to data sparsity we generated a county map for 2008. Median density was 1.56 (IQR 0.43, 3.19) for cattle and 0.007 (IQR 0.00, 0.02) for pigs, and 16,756 gHAT cases were reported. The RR was 0.59 (95% CI 0.36, 0.83) for cattle and 0.62 for pigs (95% CI 0.28, 1.28). These results are consistent with a reservoir effect for rHAT and a zooprophylactic effect for gHAT, whereby the tsetse fly vector exhibits preference for livestock. We will also present results from analyses to estimate the extent to which these effects are mediated by environmental variables downstream of livestock presence and upstream of tsetse abundance.

525

POULTRY OWNERSHIP AND GENETIC ANTIBIOTIC RESISTANCE DETERMINANTS IN THE GUT OF PRESCHOOL CHILDREN IN BURKINA FASO

Jessica M. Brogdon[1], Ali Sié[2], Clarisse Dah[2], Lucienne Ouermi[2], Boubacar Coulibaly[2], Elodie Lebas[1], Lina Zhong[1], Cindi Chen[1], Benjamin F. Arnold[1], Thomas Lietman[1], Jeremy Keenan[1], Thuy Doan[1], Catherine Oldenburg[1]

[1]*University of California, San Francisco, San Francisco, CA, United States,* [2]*Centre de Recherhe en Sante de Nouna, Nouna, Burkina Faso*

Animal to human transmission is likely a source of antibiotic resistance. Antibiotics are utilized for animal husbandry practices globally. Data from a randomized trial of pediatric antibiotic administration was secondarily evaluated to determine if poultry ownership was significantly associated with the presence of gut genetic antibiotic resistance determinants among children in Burkina Faso. Two rural communities in the Nouna District of Burkina Faso were selected based on the most recent Health and Demographic Surveillance Site census. Households with at least 2 children aged 6-59 months old were enrolled. Caregivers completed a baseline questionnaire where they reported the number of poultry owned by the household. Rectal swabs were collected from children at baseline and 5 days following antibiotic treatment. Children were treated with azithromycin, amoxicillin, cotrimoxazole, or placebo. Antimicrobial resistance determinants were classified using DNA sequencing. We measured the relationship between genetic resistance determinants and chicken ownership using a logistic regression model adjusted for confounding variables such as age and latrine type. 118 children were included in this analysis from the villages Kamadena and Dara. 90% of households reported to own at least one chicken. Children living in households reporting poultry ownership had 4 times the odds of tetracycline resistance determinants in the gut compared to those without household poultry (OR 4.14, 95% CI 1.10 to 15.6, *P*=0.04). Resistance to other antibiotic classes was common but not statistically significant between groups like beta-lactams (OR 3.11, 95% CI 0.77 to 12.6, *P*=0.11) and macrolides (OR 1.20, 95% CI 0.33 to 4.36, *P*=0.78). Poultry ownership was associated with increased odds of genetic tetracycline resistance determinants. Tetracycline is commonly used in this setting to stimulate poultry growth, which may explain these findings. Understanding the origins of antibiotic resistance may help spur the development of interventions to combat the global antimicrobial resistance crisis.

527

NOVEL HAPLOTYPES OF PFCRT IN *PLASMODIUM FALCIPARUM* FROM THE YUNNAN PROVINCE, CHINA CONFER RESISTANCE TO THE FIRST LINE ANTIMALARIAL PIPERAQUINE

Jennifer L. Small-Saunders[1], Laura M. Hagenah[1], Satish K. Dhingra[1], Kathryn J. Wicht[1], Jonathan Kim[1], Eva Gil Iturbe[1], Matthias Quick[1], Filippo Mancia[1], Paul D. Roepe[2], Margaret J. Eppstein[3], David A. Fidock[1]

[1]*Columbia University, New York, NY, United States,* [2]*Georgetown University, Washington, DC, United States,* [3]*University of Vermont, Burlington, VT, United States*

Plasmodium falciparum (Pf) malaria is a public health threat in border regions of the Yunnan Province, China due to their proximity to malaria-endemic countries. Parasites are exposed to varying, country-specific antimalarial regimens. Prior reports indicated that piperaquine resistance (PPQ-R) existed in this region and PPQ combined with dihydroartemisinin has faced widespread resistance across the Greater Mekong Subregion. Data showed that recently-emerged PfCRT mutations in this region are playing a key role in PPQ-R. Few studies, however, have examined whether *pfcrt* variants elsewhere might alter PPQ susceptibility. We used zinc-finger nuclease-based gene editing to introduce novel *pfcrt* alleles reported in the Yunnan Province into Dd2 parasites: China E (GB4 +

I371R), China B (China E +E75D/A144Y/S220A), and China C (China B + R371I). China C showed significant PPQ-R in PPQ survival assays. China E, B and C were sensitized to the former first-line drug chloroquine (CQ), despite having the CQ resistance marker K76T. China C was also sensitized to monodesethyl-CQ and quinine. Susceptibility to other first line antimalarials was not affected. Transport studies with purified China C PfCRT protein in proteoliposomes revealed elevated PPQ transport and reduced CQ transport. Molecular modeling will explore how specific mutations interact to alter PPQ and CQ susceptibilities. China B and C lines had distended digestive vacuoles in trophozoites and schizonts. Fitness assays showed that PPQ-R comes at a major fitness cost for China C. We measured dose-response of growth to PPQ and CQ of the China lines and contemporary, edited PfCRT lines (Dd2+T93S, I218F, F145I) to explore potential treatment regimens. Using evolutionary simulations on these empirically determined fitness landscapes, we show that combination therapy with PPQ and CQ may be effective in some cases. This work identifies novel PfCRT haplotypes that may be driving PPQ-R in Yunnan Province, provides insights into PPQ-R molecular transport and, more broadly, provides evidence that re-introducing CQ may be an effective rescue therapy for most PfCRT variants in regions of PPQ-R.

528

DISSECTING THE ROLE OF *PLASMEPSIN II AND III* IN PIPERAQUINE RESISTANT *PLASMODIUM FALCIPARUM* LINES

Breanna Walsh, Robert L. Summers, Sarah K. Volkman, Dyann F. Wirth, **Selina Bopp**

Harvard T.H. Chan School of Public Health, Boston, MA, United States

The spread of artemisinin (ART) resistance renders partner drugs used in ART Combination Therapies more vulnerable to emerging drug resistance. Lately, loss of efficacy of the partner drug piperaquine (PPQ) has been spreading in Southeast Asia.Major genetic determinants associated with recrudescence are increased copy numbers of *plasmepsin II* and *III* and more recently mutations in the *chloroquine resistance transporter* (*pfcrt*). While mutations in *pfcrt* were shown to be protective *in vitro*, experimental data on the role of plasmepsins in PPQ resistance is sparse. We have previously presented a bimodal growth response to increasing PPQ concentrations in PPQ resistant *P. falciparum* isolates from Cambodia in the absence of *pfcrt* mutations. We chose the area under the curve (AUC) instead of the conventional half-maximal effective concentration (EC_{50}) used in drug assays to quantify the bimodal response. To specifically determine the role of plasmepsins in the response to PPQ, we used a relevant Cambodian isolate with a duplication in *plasmepsin II* and *III* but no mutation in *pfcrt*. Using this clonal isolate, we generated *plasmepsin II*, *plasmepsin III* and *plasmepsin II/III* combination KO lines with the CRISPR/Cas9 system. Our data demonstrate that a reduction in either *plasmepsin II* or *III* decreases the AUC compared to the parental line indicating direct involvement of *plasmepsin II* and *III* in the PPQ response. We were not able to detect significant differences in hemoglobin catabolism disruption by PPQ in Fe fractions (hemoglobin, free heme, and hemozoin) of parasite lines with different *plasmepsin* copy numbers. In contrast, changing the homeostasis of the food vacuole with pH modulators (CCCP or concanamycin A) did reduce survival under high PPQ exposure. We demonstrated that increased *plasmepsin* copy number enhances survival under high PPQ pressure in Cambodian parasites and likely contributes to the emergence of resistance.

529

DEFORMABILITY OF INFECTED RBC AS THE KEY FEATURE OF PERSISTENCE AND RECRUDESCENCE IN ARTEMISININ-RESISTANT MALARIA?

Mallorie Dépond[1], Chanaki Amaratunga[2], Charlotte Chambrion[1], Lucia Angella[1], Sokunthea Sreng[3], Manel Ouji[4], Lucie Paloque[4], Aurélie Fricot-Monsinjon[1], Ilhame Tantaoui[5], Sivanna Mao[6], Chantha Sopha[7], Baramey Sam[8], Kasia Stepniewska[9], Seila Suon[3], Sylvestre Biligui[5], Philippe Guerin[9], Jérôme Cros[10], Béatrice Aussilhou[10], Safi Dokmak[10], Jean-Michel Augereau[4], Françoise Benoit-Vical[4], Rick Fairhurst[2], Pierre Buffet[1], Papa Alioune Ndour[1]

[1]*INSERM U1134 INTS. Labex GRex, Paris, France,* [2]*Laboratory of malaria and vector research NIH, Rockville, MD, United States,* [3]*National Center for Parasitology, Phnom Penh, Cambodia,* [4]*LCC-CNRS, Toulouse, France,* [5]*INSERM-Cimi U1135, Paris, France,* [6]*Sampov Meas Referral Hospital, Pursat, Cambodia,* [7]*Makara 16 Referral Hospital, Preah Vihear, Cambodia,* [8]*Ratanakiri Referral Hospital, Banlung, Cambodia,* [9]*Infectious diseases data observatory WWARN, Bangkok, Thailand,* [10]*APHP Beaujon, Paris, France*

Progress towards malaria elimination achieved using artemisinin derivatives (ART) is threatened by *P. falciparum* resistance, characterized by delayed clearance of infected RBC (iRBC) and recrudescence, correlated with specific mutations in the parasite *Kelch13* gene. The mechanisms of slow parasite clearance are still not fully elucidated. We hypothesize that ART-resistant iRBC escape retention and clearance in the spleen by maintaining deformability compared to sensitive strains. Clearance kinetics of iRBC and pitted RBC was quantified in 88 patients at 3 Cambodian sites. An *ex vivo* human spleen model was used to perfuse simultaneously ART sensitive (F32 TEM) and ART resistant (F32 ART) to evaluate clearance dynamics and phenotypic changes. Finally, the specific deformability of each population was evaluated by enrichment (>90% of iRBC) of the two strains with streptolysin-O (SLO). Deformability was assessed by ektacytometry. In patients with parasite clearance half-life greater than 5 hours, appearance of pitted RBC was also delayed (p<0.001). Upon *ex vivo* perfusion of human spleens with the two strains exposed to ART, clearance of F32 TEM was faster than that of F32 ART. A plateau of clearance was reached after 30 min of perfusion where 5% of F32 TEM remain in circulation vs 20% in F32 ART (n=4). At 180 min, the F32 TEM showed a higher pitting rate than the F32 ART (37% vs 28%) (n=2) and a higher parasite clearance rate (98.3% vs 90.8%). The normalized circulating parasite biomass was 5.6 times higher in F32 ART compared to F32 TEM and recrudescence assay confirmed growth of only F32 ART. Finally, deformability index (EI) measured by ektacytometry in concentrated ART-exposed iRBC showed that RBC hosting the F32 TEM clone were less deformable than RBC hosting the F32 ART (median EI =0.371 vs 0.424 respectively from 2 perfused-human spleens). Taken together, these results suggest that ART-resistant iRBC remain in circulation by maintaining their deformability during quiescence, explaining the lower splenic retention and delayed clearance of AS-resistant strain. Explorations to decipher the key players of this phenotype are ongoing.

530

MUTATIONS IN A PUTATIVE LYSOPHOSPHOLIPASE ARE ASSOCIATED WITH ALTERED EX VIVO SUSCEPTIBILITY TO MULTIPLE ACT PARTNER DRUGS

Melissa D. Conrad[1], Ozkan Aydemir[2], Patrick K. Tumwebaze[3], Oswald Byaruhanga[3], Martin Okitwi[3], Stephen Orena[3], Roland A. Cooper[4], Jeffrey A. Bailey[2], Philip J. Rosenthal[1]

[1]*University of California, San Francisco, CA, United States,* [2]*Brown University, Providence, RI, United States,* [3]*Infectious Diseases Research Collaboration, Tororo, Uganda,* [4]*Dominican University of California, San Rafael, CA, United States*

Malaria control is challenged by resistance to artemisinins and artemisinin-based combination therapy (ACT) partner drugs, as seen in Southeast Asia. Although first line ACTs remain effective in Africa, worrying trends suggest resistance may be on the way, making monitoring for changes

in drug sensitivity and efforts to identify resistance mediators important. To search for associations between drug sensitivity phenotypes and *P. falciparum* genotypes, we used a molecular inversion probe (MIP) platform to sequence 45 genes, including 27 known or predicted mediators of drug resistance. We genotyped 235 isolates from eastern Uganda (2015-18) with ex vivo drug sensitivity data. We combined Wilcoxon signed-rank tests ($p < 0.01$) and random forest machine learning (RF) approaches (top 10 variable importance estimates in ≥ 2 of 5 simulations) to explore relationships between ex vivo IC_{50}s and SNPs in targeted genes. Validating our approach, *PfCRT* mutations associated with chloroquine resistance (76T, 74I, 75E, 271E, 371I) were, compared to wild type, significantly associated with higher chloroquine IC_{50}s, and were among the most important variables to explain IC_{50} variation in RF models. We identified SNPs in other genes that may mediate changes in drug sensitivities. SNPs in PF3D7_0218600 were associated with decreased sensitivity to lumefantrine (4.0 nM WT vs 11.8 nM Mix/Mut, $p = 0.001$), but increased sensitivity to mefloquine (8.2 nM WT vs 3.2 Mix/Mut nM, $p = 0.002$) and piperaquine (4.8 nM WT vs. 3.3 nM Mix/Mut, $p = 0.001$). Mutations in this gene, predicted to encode a lysophospholipase, were previously selected *in vitro* with primaquine, suggesting that loss of function may lead to degradation or activation of antimalarials intracellularly. In addition, we found novel mutations in PfMDR1 associated with altered sensitivity to lumefantrine and mefloquine, a mutation in PfMDR2 associated with increased sensitivity to pyrimethamine, and a mutation in a predicted transcription factor associated with increased sensitivity to pyronaridine. Our MIP approach identified multiple potential mediators of altered sensitivity to ACT partner drugs.

532

ANTIMALARIAL EFFICACY DATA ARE ROUTINELY MISREPORTED IN SUB-SAHARAN AFRICA

Mateusz Plucinski[1], Ian Hastings[2], Leah Moriarty[1], Meera Venkatesan[3], Ingrid Felger[4], Eric S. Halsey[1]

[1]*CDC, Atlanta, GA, United States,* [2]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [3]*USAID, Washington, DC, United States,* [4]*Swiss Tropical and Public Health Institute, Basel, Switzerland*

Antimalarials, in particular artemisinin-based combination therapies (ACTs), are critical tools in reducing the global burden of malaria. Antimalarial efficacy studies indicate that ACTs remain highly efficacious in Sub-Saharan Africa, unlike other regions such as Southeast Asia. Performing and reporting antimalarial efficacy studies in a transparent and standardized fashion permits efficacy outcomes to be compared across countries and time periods. A key component of antimalarial efficacy studies is molecular correction, where recurrent parasitemias are classified as new infection or recrudescence to obtain a final corrected efficacy outcome. In order to evaluate how well studies from sub-Saharan Africa adhered to these guidelines, we reviewed 273 articles where ACT efficacy was a reported outcome. Molecular correction was used in 84% (228/273) to distinguish new infections from recrudescences in subjects experiencing recurrent parasitemia, but only 42% (95/228) of these articles provided explicit details on how molecular correction was performed. Even fewer, 3% (6/228), included genotyping data in their article or supplementary information. Only 45% (99/219) of therapeutic efficacy articles performing molecular correction reported corrected efficacy outcomes calculated in a way consistent with WHO recommendations. In all the articles reviewed, only 9% (25/273) provided a statement on data availability. These results indicate data insufficiency and potential widespread biases in how therapeutic efficacy data are being reported from Sub-Saharan Africa. Increased transparency and comparability between antimalarial efficacy studies will be achieved when investigators provide clear descriptions of their methodology, present detailed molecular correction data for each subject experiencing a recurrent parasitemia, calculate Kaplan-Meier estimates of corrected efficacy, and adhere to WHO laboratory and reporting guidance.

534

UNDERSTANDING RELIGIOUS PERSPECTIVES ABOUT DEATH AND SAMPLE COLLECTION FROM DEAD BODIES USING MINIMALLY INVASIVE TISSUE SAMPLING IN THE CONTEXT OF THE CHAMPS STUDY IN HARAR AND KERSA: EASTERN ETHIOPIA.

Ketema Degefa Begna[1], Mohammad Aliyi[1], Berhanu Damise[1], Azeb Kidane[1], Nega Assefa[1], Lola Madrid[2]

[1]*Haramaya University, Ethiopia, Harar, Ethiopia,* [2]*London School of Hygiene & Tropical Medicine, London, United Kingdom*

Religious leaders are the key facilitators for burial rituals. Religious beliefs about handling the deceased body and life after death affect sample collection. Religious leaders are called on to provide spiritual support to help families in the grievance. This study tries to understand religious perspectives that should be considered while planning for MITS. Seventy-five participants who are key people in relation to death and grievance took part in the interview. Twelve in-depth interviews, six semi-structured interviews, six focus group discussions and nineteen participant observations were performed between September 2017-February 2019. Participants believe that after a child dies their soul leaves the body, becomes an independent entity, and is capable of conducting religious function. The need to respect the dead body is emphasized. Muslim beliefs do not permit removing any part of a dead body. Participants believe religious leaders can facilitate sample collection from dead bodies using minimally invasive tissue sampling (MITS). Christian participants explain at death the body returns to dust while the soul returns to the creator, God. Both Muslim and Christian participants explain death is the will of God. Muslim objections to MITS were based on the importance given to the body at death; the MITS procedure will delay burial, undermine the sacredness of the body, and inflict pain on the body, which the child will continue to feel until burial. Understanding the role of religion in collecting tissue samples has practical implications for health practitioners; especially during the processes for consent and family follow up.

535

DESCRIPTIVE ANALYSIS OF VACCINE TRENDS IN THE DOMINICAN REPUBLIC: IS THE D.R. UNAFFECTED BY THE VACCINE HESITANCY MOVEMENT?

Miguel Delgadillo[1], Alejandro Villanueva[2], Priscila Abate[2], Jose Duran[2], Paola Peña[2], Yarimar Pérez Del Leguas[1], Santos García Vázquez[1], Laura Manosalvas[2], Leandro Tapia[2], Robert Paulino-Ramirez[2]

[1]*School of Medicine, Universidad Iberoamericana (UNIBE), Santo Domingo, Dominican Republic,* [2]*Institute for Tropical Medicine & Global Health, UNIBE, Santo Domingo, Dominican Republic*

In 1974 the World Health Organization summoned all the countries in the world to establish a Government Immunization Program, in which vaccines were going to be provided for six diseases: tuberculosis, polio, diphtheria, bordetella pertussis, tetanus, and measles. During the last years, there has been a decrease in vaccinations and it has taken a toll on disease outbreaks like diphtheria in the years 2018-2019, however in Latin America vaccination rates haven't shown any change. During the past years, emerging and re-emerging diseases have caused high economic burden in the region, making it essential to strengthen the preventive programs in place. The aim of this study is to describe the vaccination trend in the Dominican Republic from 2012-2019. Data of Vaccination Records within the Governmental Immunization Program were solicited to the Ministry of Health through its online platform. Information of Vaccine Preventable Diseases were extracted from the Ministry of Health Weekly Reports. Vaccine scheme completion for the Pentavalent vaccine during the studied period increased from 85% coverage in 2012 to 90% in 2019, with the lowest coverage in 2013 with 82.6%, and the highest with 93.6% in 2018. *S. pneumococcus* vaccine coverage during the studied period increased from 20.8% coverage in 2013 to 93.2% in 2019 without data for 2012. Vaccine completion for MMR vaccine increased

from 87.7% in 2012 to 94.3% in 2019, lowest coverage was reported at 83.4% in 2013. Pentavalent vaccine HIT milestones were successful for *C. diphtheriae* but failed to achieve full *B. pertussis* coverage until 2018. This vaccination trend could explain the low diphtheria circulation in the nation and recurrent outbreak of *B. pertussis*. For *S. pneumoniae* coverage, the Pneumococcus-C vaccine successfully reached the HIT milestones in 2013. The MMR vaccine HIT milestones have been successful since 2012 for Rubella, and reached the milestones for Measles in 2018. Public health officials should continue to monitor vaccination efforts and vaccine preventable diseases incidence.

536

"BATAKA TWETAMBIRE" (NATIVES, WE HEAL OURSELVES): REMOVING BARRIERS TO HEALTHCARE ACCESS FOR THE SEVERELY DISADVANTAGED IN UGANDA

Benjamin B. Norton[1], Scott Kellermann[2], Michael C. Borecky[3], Thomas E. Borecky[3], Birungi Mutahunga[4], Nahabwe Haven[4], Latha Rajan[1]

[1]*Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States,* [2]*Fulbright Scholarship Program, Kanungu, Uganda,* [3]*Loma Linda University School of Medicine, Loma Linda, CA, United States,* [4]*Church of Uganda Bwindi Community Hospital, Kinkizi Diocese, Kanungu, Uganda*

Responding to the acute needs of the impoverished and remote populations in Uganda, the Bwindi Community Hospital (BCH) was established in Kanungu, Uganda. Health care can be economically devastating in rural Uganda, especially to the indigenous Batwa pygmies who survive on <$0.80 /day/family. In 2010, BCH launched a community-based health insurance called eQuality Health Insurance (eQHI). Currently, >28,000 individuals (40% of BCH's catchment area) are insured, although Uganda's average enrollment in any CBHI is <2% population. In order to design strategies to leverage social capital and remove barriers, we conducted a needs assessment survey to identify barriers. Using a random number generator, 10 clusters of 50-70 households were chosen, and individual households randomly selected. Women in the households were mobilized using local leaders and cash incentives. A total of 219 women were surveyed in focus groups. Surveyed women cited cost and distance as the highest barriers to both receiving treatment (92.2%, 89.5%) and eQHI enrollment (93%, 92%). Lower eQHI coverage was associated with low household education (35.7% vs. 54.8%; p<0.005), inability to afford eQHI (31.4% vs. 50.0%; p<0.005), losing money with eQHI (13.1% vs. 60.4%; p<0.0001), and living >3 hours walking distance from BCH (35.7% vs. 53.2%; p<0.05). Having eQHI was associated with higher rates of utilization of BCH treatment services for malaria (94.8% vs 50.0%, p<0.0001), maternal health (99.0% vs 51.2%, p<0.0001), vaginal births (77.9% vs 52.1%, p<0.0001), and all deliveries (98.1% vs 37.4%, p<0.0001). Living < 3 hours walk of BCH was associated with increased treatment rates for malaria (74.5% vs 61.2%; p<0.05), but not maternal services (70.9% vs. 63.9%; p>0.05) or deliveries (67.6% vs. 68.0%; p>0.05). Costs and distance are the two major barriers to both receiving care and enrollment in eQHI. As of 2019, eQHI is $5/person/year with an $0.80 copay at time of service, but it is completely free for families subsisting on <$0.80/day. Strategies to reduce or remove distance barriers are important steps towards universal healthcare coverage in this marginalized population.

537

SHARING THE SAME GEOGRAPHY - USE OF A COMMON-GEOREGISTRY AS A CENTRAL AUTHORITY FOR GEOGRAPHIC INFORMATION CORE TO DISEASE SURVEILLANCE AND RESPONSE

Anne Liu[1], Abigail Ward[1], Pedro Pagalday Olivares[1], Julia Dunn[1], Sameen Babur[1], Lakshmi Balachandran[1], Steeve Ebener[2], Izay Pantanilla[2], Nathan McEachen[3], Justin Lewis[3], Derek Treatman[4], Scott Teesdale[4], Arnaud Le Menach[1]

[1]*Clinton Health Access Initiative, New York, NY, United States,* [2]*Health Geolab, Manila, Philippines,* [3]*Terraframe, Denver, CO, United States,* [4]*Vital Wave, Palo Alto, CA, United States*

Geography and time are critical dimensions for monitoring the health of populations, ensuring populations have access to care, and formulating public health policies. The usefulness of tools for geographic analysis can be hindered by non-standardized and changing geographic attributes, missing GPS locations, and undocumented historic changes. Further, in the absence of trusted geographic sources, stakeholders develop their own databases, leading to misaligned or duplicate datasets. The ripple effect of low quality or fragmented geographic information can be widespread, resulting in inequitable or incomplete delivery of health services.The Common Geo-Registry (CGR) is a novel, open-source platform that ensures the availability of up-to-date, standardized, geographic reference information for information systems. The CGR allows simultaneous hosting, management, regular update, and sharing of master lists of geographic objects (e.g. health facilities, villages, administrative divisions), hierarchies, and associated geospatial data through time. Data can be downloaded from the CGR for use in a Geographic Information System (GIS) or accessed by program-specific systems to enable proper contextualization of program data, facilitate trend analysis, aggregate data according to different hierarchies, and support the creation of informative maps. Implementation began in early 2020 with assessments in Southeast Asia and Southern Africa. The assessment process includes interviews with government stakeholders and review of current geographic information. Results will determine the steps necessary for a CGR deployment, which may include strengthening of the governing structures, generation of master lists, hierarchies, and associated geospatial data, technical capacity strengthening, and integration with existing information systems - including those from other sectors. Using a common geography is expected to improve disease surveillance processes and may be applied in other public health settings. To that end, deployments will be monitored to assess the use of CGR on analyzing disease trends across time and space.

538

MEASURING PERCEIVED QUALITY OF CARE TO INCREASE UTILIZATION FOR BETTER PERFORMANCE OF A MALARIA SURVEILLANCE SYSTEM IN SENEGAL

Ashley Garley[1], Roger Tine[2], Moustapha Cisse[3], Medoune Ndiop[3], Mame Birame Diouf[4], Katharine Sturm-Ramirez[5], Yazoumé Yé[1]

[1]*ICF International, Rockville, MD, United States,* [2]*Université Cheikh Anta Diop de Dakar, Dakar, Senegal,* [3]*Programme National de Lutte contre le Paludisme, Dakar, Senegal,* [4]*United States Agency for International Development, Senegal and U.S. President's Malaria Initiative, Dakar, Senegal,* [5]*Centers for Disease Control and Prevention, Atlanta, USA and U.S. President's Malaria Initiative, Dakar, Senegal*

Ensuring use of health care services is crucial to detect malaria cases coming into a country's health care system and to the improvement of a malaria surveillance system. To assess system performance in Senegal, we used a mixed-methods approach in three regions selected using National Malaria Control Program-defined malaria transmission zones stratified by annual incidence (Low <5/1000, Moderate 5-25/1000, and High >25/1000). The perceived quality of care component included exit interviews with 540 patients who received services for fever at health facilities (HF). Four dimensions were assessed: health personnel practices and behavior; adequacy of resources and services; healthcare delivery;

and accessibility, using verbatim statements and a five-point Likert scale. Responses were re-coded as scalar values from -2 to +2, neutral being 0. Negative 2 represented low satisfaction and +2 represented high satisfaction. Mean scores were then calculated for each statement and dimension. Overall, patients were satisfied with the quality of care received (overall 1.24), particularly health personnel practices and behavior (1.30-1.50) and health service delivery (0.78-1.10). Respondents were less favorable about adequacy of resources and services (0.59-1.10), mainly due to the unavailability of certain malaria drugs (0.59). Least favorable was accessibility, which comprised financial and geographical barriers to access health care (0.22-0.69). Affordability (0.60), access to drugs (0.69), and the distance to get to a HF (0.22) were not satisfactory. Comparing transmission zones, patients in the moderate zone were the most satisfied across all dimensions, except for a low rating on the distance between HFs (0.14). Patients in the low transmission zone reported the least satisfaction in health service delivery. Patients in all zones were generally pleased with the services they received, a rating that should encourage future use of services. Investigating further the issues on drug availability and accessibility to understand concerns and resolve them will safeguard use of HFs to detect cases and lead to improved system performance.

540

COMMUNITY PERCEPTIONS, BELIEFS AND PRACTICES AROUND STILLBIRTH AND <5 CHILD DEATHS: AN EXPLORATORY STUDY IN A RURAL SETTING, BANGLADESH

Sazzad Hossain Khan[1], Tonmoy Sarkar[1], Md. Saiful Islam[1], Hossain MS Sazzad[2], Syeda Nurunnahar[1], Md. Musa Baker[1], Mohammad Ariful Islam[1], Kamal Ibne Amin Chowdhury[1], Dalia Yeasmin[1], Faruqe Hussain[1], Shams El Arifeen[1], Khatia Munguuambe[3], John Blevins[4], Emily S. Gurley[5], Shahana Parveen[1]

[1]*icddr,b, Dhaka, Bangladesh,* [2]*Kirby Institute, New South wales, Australia,* [3]*Centro de Investigação emSaúde de Manhiça, Manhica, Mozambique,* [4]*Emory Global Health Institute, Atlanta, GA, United States,* [5]*John Hopkins University, Baltimore, MD, United States*

Bangladesh has static rates of neonatal and infant mortality with a slow progress in declining <5 child during 2014-2018. Limited studies investigated local interpretation of child deaths to inform prevention initiatives. We explored community members' beliefs, perceptions and practices around stillbirth and <5 child deaths to support the implementation of minimally invasive tissue sampling (MITS), a post-mortem procedure to determine the cause of stillbirth and <5 child death at Baliakandi sub-district, Bangladesh. We conducted 37 key informant interviews, 7 focus group discussions and 6 semi-structured interviews with family members of deceased children, community and religious leaders, those who prepared bodies for burial and healthcare providers in two phases between April 2017-April 2018. We coded interviews and performed thematic analysis. The respondents believed death is predestined and happens when God wishes. Stillbirth was considered an outcome of disobeying or not performing religious rituals properly by parents. Pregnant women and newborns were perceived to be vulnerable as they could easily be possessed by evil spirits, which are believed to be capable devouring the fetus and child. Several practices for pregnant and newborn mothers were considered causes of child loss. During pregnancy, a heavy workload and excessive intercourse were reported as factors for stillbirth. Early marriage, preference for home delivery, delayed care seeking and mistrust of the physician were reported to be critical for child death. Respondents identified pneumonia, diarrhea, Nipah, and congenital diseases as known causes of child death. Drowning and 'fear' among <5 children were also reported as common causes of death. The study identified that respondents perceived stillbirth and <5 child death are linked to individual behaviors and supernatural power that resulted in poor health seeking behavior that may further affect the MITs participation. Interventions that target improving villagers knowledge and perception regarding stillbirth/ <5 child death may improve health seeking behavior and reduce child mortality.

541

EVALUATING THE INFLUENCE OF ASYMPTOMATIC SLEEPING SICKNESS INFECTIONS ON INTERVENTION PROGRAMS AND ELIMINATION GOALS USING MATHEMATICAL MODELLING

Maryam Aliee[1], Ron E Crump[1], Erick Mwamba Miaka[2], Matt J Keeling[1], Kat S Rock[1]

[1]*University of Warwick, Coventry, United Kingdom,* [2]*Programme National de Lutte contre la Trypanosomiase Humaine Africaine, Kinshasa, Democratic Republic of the Congo*

Sleeping sickness (gambiense human African trypanosomiasis, gHAT) is a vector-borne disease transmitted to humans by tsetse, mainly in Western and Central Africa. It is one of the neglected tropical diseases and the global elimination of transmission is set as a 2030 goal by the World Health Organization following the success of recent active and passive screening programmes. There is evidence of asymptomatic infection of gHAT, however, there is uncertainty of its role in transmission and maintenance of the disease. In this research study, we would like to address how asymptomatic infection may influence elimination. Mathematical models modulated by the available data have been increasingly used in recent years to study the dynamics of infection and investigate strategies to reach elimination goals of sleeping sickness. Based on the established framework, we develop a minimal model to account for asymptomatic populations with self-cure capabilities and different transmission rates. Given current screening and treatment protocols, some types of asymptomatic gHAT infections can be diagnosed and treated, in contrast to many other infections. On the other side, some asymptomatic cases, such as the ones with skin-only infection, may be misdiagnosed by the typical diagnostic algorithms. We estimate the parameters of self-cure rate and the relative detection probability based on analogous available gHAT studies. We then fit our model to human case incidence data recorded at the health zone level across the Democratic Republic of Congo (DRC) to estimate the free parameters of this modified model. This allows us to study various intervention strategies and analyse the impact of the asymptomatic infection on the disease dynamics by comparing to the results of the previous versions of the model without explicit inclusion of asymptomatics. More importantly, we examine how asymptomatic individuals may impact the 2030 goal of elimination of transmission under different intervention programmes. This will shape our recommendations for effective elimination strategies.

542

THE APPLICATION OF PARTICIPATORY, EPIDEMIOLOGICAL-ECONOMIC MODELLING FOR POLICY GUIDANCE: THE CASE OF PERTUSSIS VACCINATION IN SOUTH AFRICA

Rachel A. Hounsell, Rudzani Muloiwa, Benjamin Kagina, Sheetal P. Silal

University of Cape Town, Cape Town, South Africa

Mathematical modelling of infectious diseases provides a tool for policy makers and public health planners to predict the impact and cost-effectiveness of possible intervention strategies. While such models are often considered difficult to interpret for non-experts, there are novel approaches (such as interactive web-based dashboards, data visualization techniques and participatory modelling) for collaboratively building and communicating modelling processes and results to improve the applicability and acceptability of modelling to inform real-world public health policy decisions. Pertussis is a highly contagious, vaccine-preventable respiratory tract disease. Despite well-established vaccination programmes, pertussis persists as a major public health concern. A growing body of evidence recommends supplementing childhood vaccination with strategies such as targeted booster doses and maternal immunization to tackle this burden. However, minimal research has been conducted in low- and middle-income countries, where the need is greatest. We developed an age-stratified, stochastic compartmental transmission model to predict the impact and cost-effectiveness of maternal and booster vaccination strategies on the burden of pertussis in

South Africa. We used an iterative participatory approach where diverse stakeholders were involved in the model building and parameter selection process. Based on this model, we demonstrate the opportunity of creating an interactive, visual, web-based application of model scenarios and outputs to support evidence-based policy making. The use of participatory modelling and interactive processes encouraged ownership, allowed us to incorporate different perspectives, and strengthened the robustness and relevance of the model and its projections. The research forms part of an ongoing collaboration between several national policy, clinical and academic groups in South Africa.

543

ENGAGING YOUNG PEOPLE AS AGENTS OF CHANGE: A PRIMARY SCHOOL EDUCATIONAL INTERVENTION TO DECREASE ARBOVIRAL AND PROTOZOAL DISEASE RISK IN GRENADA

Trevor Noel[1], Jeffon Telesford[1], Victor Ashby[1], Jonathan Altamirano[2], Nandy Noel[1], Nikita Cudjoe[1], Sherice Phillip[1], Connie Dottin[3], Francis Hector[3], Kennie James[3], Paul Fields[1], Calum Macpherson[1], A. Desiree LaBeaud[2]

[1]*Winward Islands Research and Education Foundation, St. George's University, St George, Grenada,* [2]*Stanford University, Stanford, CA, United States,* [3]*Ministry of Health, St George, Grenada*

School-based educational programs have shown to be effective for environmental health risks, such as arboviral and protozoal diseases. This randomized controlled trial seeks to determine whether an educational intervention can produce long-term improvements in knowledge, attitudes, and practices regarding these diseases, among grade 4 primary school students in Grenada. Forty (40) primary schools were randomized to either receive a two-day educational intervention (n=17; 487 children) or to be a control (n=23; 578 children). The intervention included an oral presentation, interactive demonstrations, and informational posters. Pre- and post-intervention questionnaires were administered before and after the intervention and evaluated participants' knowledge of disease and prevention of feco-orally transmitted protozoan diseases and arboviral diseases and self-efficacy in disease prevention. Participants answered similar questions with a fair level of agreement (Kappa coefficient of 0.382). The intervention group reported significant improved arboviral knowledge ($p<.0001$), attitudes ($p<.0001$), and a 27% increase in answering: "Where do *Aedes aegypti* female mosquitoes lay their eggs?" correctly between tests. Intervention participants also reported improved feco-oral disease knowledge ($p<.0001$), attitudes ($p<.0001$), and a 6% increase in answering: "What is the best way to protect yourself from feco-orally transmitted germs?" correctly between tests. Intervention participants also improved self-efficacy ($p< .0001$), By comparison, the control group showed significant improvements in feco-oral disease knowledge ($p<.0001$) and attitudes ($p<.0001$), arboviral disease attitudes ($p<.0001$) and self-efficacy ($p<.0001$), but not arboviral disease knowledge ($p= .8643$) between tests. Next steps include six month and one-year evaluations of knowledge, attitudes and self-efficacy to determine whether improvements persist.

544

CAN ELIMINATION OF SLEEPING SICKNESS BE COST-EFFECTIVE? AN ECONOMIC EVALUATION OF GHAT ELIMINATION CAMPAIGNS IN THE DRC

Marina Antillon

Swiss Tropical and Public Health Institute, Basel, Switzerland

Gambiense human African trypanosomiasis (gHAT) is marked for elimination of transmission (EOT) by 2030, but the disease persists in several low-income countries. We examine the comparative efficiency of gHAT elimination strategies in the Democratic Republic of Congo (DRC), the country with the highest burden of gHAT. We compared four strategies against gHAT by coupling a transmission model of health zone epidemiology with a health outcomes and cost model. Alongside testing in fixed health facilities -- known as passive surveillance (PS) -- the strategies included mass testing in villages by mobile units -- known as active screening (AS) -- and optionally, vector control (VC) to reduce the population of tsetses, the sole vector for the parasite. A scale-back algorithm was simulated for AS and VC when no cases were simulated for three years. Elimination by 2030 appears feasible in most settings, but could require additional coverage of AS or VC in most medium-to-high risk locations. Costs of gHAT strategies are primarily driven by AS and, if used, VC. The scale-back of AS and VC means most investments (over 80%) for the next two decades will be made by 2030 if the more efficient strategies are chosen. In low-risk settings, minimum cost strategies consist of AS and PS and lead to EOT by 2030 with high probability. For high-risk settings, strategies including VC could be cost-saving while ensuring EOT quickly. In moderate-risk settings the choice of strategy depends on the objective; medical-only strategies (AS and PS) appear cost-effective for low-to-moderate willingness-to-pay (WTP) to avert DALYs ($0-500/DALY), but have low probability of achieving EOT by 2030. High-coverage AS or VC could be cost-effective for a higher WTP (over $1000/DALY) and these strategies are more likely to lead to EOT. In many settings, the goal of EOT by 2030 is a sensible use of resources and comparable to the costs of efforts to eliminate other pathogens.

545

INTERSECTIONALITY AND HEALTH-RELATED STIGMA: A QUALITATIVE STUDY IN INDONESIA

Ruth Peters

Vrije University Amsterdam, Amsterdam, Netherlands

Health-related stigma is a complex phenomenon and important in the context of tropical medicine. The experience intersects with other adversities that arise from a diversity of social inequalities and oppressive identities like gender, sexuality, and poverty. Therefore, we see promise in embedding the concept of 'intersectionality' into the theory of health-related stigma. The main objective of this paper is to build upon the concept of intersectionality in health-related stigma by exploring the convergence of experiences of stigma and other adversities among people living with stigmatized health conditions in Indonesia. During this qualitative study 40 people affected by either HIV, leprosy, schizophrenia or diabetes were interviewed between March and June 2018. The study sites were Jakarta and West Java. Data was analyzed thematically following an integrative inductive-deductive approach. The mean age of the participants was 40.9 years (SD 11.54; range 19 to 75 years) and the mean duration of living with the health condition was 10.4 years (SD 5.8; range 1 to 25 years). 36 respondents reported stigma experiences owing to their health condition. The main reported intersectional inequalities were gender and poverty (n=21), followed by religion (n=13), age (n=11), co-morbidity (n=9), disability (n=6), and sexuality (n=4). While adversities related to religion and age were experienced in the macro and meso levels, individual's religious identity and age were both found to have positive impact on lives in the micro level. Those who self-reported of having a co-morbidity or disability, or identified as a sexual minority (gay or transgender), also reported of adverse experiences that intersected with that of health-related stigma. In conclusion, this study uncovered how the experience of health-related stigma intersects with other oppressions in an individual's life. The findings highlight the importance of acknowledging and understanding the multi-dimensional aspect of lives of people living with stigmatized health conditions, and warrant integrated multi-level and cross-cutting stigma reduction interventions.

546

ETHICAL ISSUES AND CHALLENGES FACED BY GLOBAL HEALTH PROGRAM DIRECTORS

Michelle Aniko Grek[1], David Addiss[2], Ashley Graham[2], Susan Landskroener[1], James V. Lavery[1]

[1]*Rollins School of Public Health, Emory University, Atlanta, GA, United States,* [2]*The Task Force for Global Health, Decatur, GA, United States*

Failure to address ethical challenges in global health programs (GHP) can reduce their impact or undermine their viability altogether. To explore how global health leaders experience, engage with, and attempt to resolve ethical challenges that arise in their programs, we conducted an open-ended qualitative survey using in-depth interviews with seven global health program directors and two senior leaders (CEO and COO) at the Task Force for Global Health (TFGH) in Decatur, GA, USA. The goals of our analysis were to describe a taxonomy of prominent ethical challenges, and to explore the various ways that the respondents identify and manage these challenges in their daily work. We identified ten categories of ethical challenges: ethical misalignment between funders, implementation and host country partners; funding and budgets as constraints on ethical decision-making; concerns about limited impact of programs improving host country capacity; concerns about missed opportunities to benefit host country communities; ethical shortcomings of current guidance and practice conventions; data governance, stewardship and management issues; challenges navigating complex sociocultural contexts; ethical challenges related to photography; reputational risks and challenges related to maintaining program trustworthiness; and accountability for unintended consequences. Program directors described their experiences of "moral ambiguity"— uncertainty about the most ethical course of action—which at times led to anxiety, and even distress, for them and their teams. Ethical guidance in global health tends to emphasize the application of abstract ethical principles. Our findings suggest that such an approach provides inadequate guidance for GHP managers and practitioners in their day-to-day decision-making. Developing a clearer picture of the ethical challenges faced by highly experienced directors of major GHP is an important first step towards understanding the gaps of the current ethics paradigm, illuminating a pathway to address these current failings and improving the integration of ethical guidance with program management.

547

HOW REAL-TIME DEATH NOTIFICATIONS FROM THE COMMUNITY HAVE BEEN USED TO IMPLEMENT MINIMALLY INVASIVE TISSUE SAMPLING IN BALIAKANDI, BANGLADESH

Abdullah Al Masud[1], Emily S. Gurley[2], Muhammad Faruqe Hussain[1], Sanwarul Bari[1], Farzana Islam[1], John Blevins[3], Ahoua Kone[3], Kyu Han Lee[2], Qazi Sadeq-ur Rahman[1], Atique Iqbal Chowdhury[1], Palash Mutsuddi[1], Shakhor Blaise Gomes[1], Abdus Suban Mulla[1], Afroz Zahan[1], Probin Kumer Dey[1], Shams El Arifeen[1], Shahana Parveen[1]

[1]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh,* [2]*John Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [3]*Rollins School of Public Health, Emory University, Atlanta, GA, United States*

The Child Health and Mortality Prevention Surveillance (CHAMPS) is ongoing in Baliakandi, a rural sub-district in Bangladesh, aiming to identify the cause of stillbirths and <5 child deaths by using minimally invasive tissue sampling (MITS), a postmortem procedure using a needle. MITS must be conducted within 24 hours of death and before burial. Since 58% of stillbirths and <5 child deaths occurred in the community and the cause of those deaths are only imprecisely examined through verbal autopsies, from June 2019, CHAMPS has been approaching for MITS of those families whose children died in the community. Getting death notification within hours is crucial so that the team can reach the household of a deceased child and approach the guardian to participate in the MITS procedure. To obtain the objective of receiving rapid notifications, we trained 1,158 community volunteers from 261 villages to notify deaths to a CHAMPS hotline number. In addition, data collectors visit households once every two months to collect health-related information and identify stillbirths and deaths. The death notification is recorded in a web-based system that sends SMS to the CHAMPS team if the death occurred within the past 24 hours. From June-December 2019, we received 80 death notifications within 24 hours, among which 42 were received within 5 hours of the deaths. After receiving SMS, the team immediately reached the households of 12 deceased children. We approached 11 families for MITS participation and 4 gave consent for MITS. The remaining 7 families refused consent for the MITS but consented to participate in clinical data abstraction and verbal autopsy. We could not approach 1 family for MITS as the body was taken outside of the surveillance area. In the other 30 deaths, the major reasons for not being able to reach the deceased's household included death notifications received after evening; ritual bathing or burial process started and bodies were taken outside surveillance area. The real-time notification system identified 40% of deaths in the community within 5 hours. This can be more effective by periodical performance evaluation and by executing necessary actions.

548

RAPID RESPONSE PLATFORM TO MANUFACTURE HUMAN IMMUNOGLOBULIN PRODUCT

Evelyn Van der Hart, Aynslie Wall, Patrick Wiebe, Grayson Poyser, Russell Pronyk

Emergent BioSolutions, Winnipeg, MB, Canada

As seen with the recent COVID-19 outbreak, the potential threat of infectious diseases for rapid loss of lives globally is increasing. A rapidly deployable drug product for use in the early stages of an outbreak could prevent further spread of the pathogen to an at-risk population, such as health personnel. Emergent BioSolutions is developing a rapid response platform (RRP) to manufacture human immunoglobulins (HIG) for use as passive immunotherapy during public health emergencies. Emergent's RRP is being developed to address the need for front-line therapy/prophylaxis with immediate benefit with the potential to overcome the current limitations of convalescent plasma. Emergent's proposed RRP begins with plasma donor identification using a field deployable real-time donor screening assay to measure levels of pathogen-specific antibodies. Plasma collected from eligible donors is treated using the Mirasol® Pathogen Reduction Technology, pooled, purified and concentrated into HIG using Emergent's rapid HIG manufacturing process to produce product formulated for intramuscular administration. Emergent has designed and constructed a working modular manufacturing unit (MMU) prototype that is equipped to run the rapid HIG manufacturing process, providing a logistical advantage with potential for plasma collection and manufacturing to both occur in an impacted region. The MMU consists of a modified shipping container to meet ISO 8 environment requirements with self-contained utilities, including HVAC, and can run off a diesel generator or local power. Our rapid HIG process aims to purify, formulate, and fill ~60 doses (1 g of total IgG) from 30L of source plasma with testing and release onsite for immediate use. Multiple innovations to streamline operations, including single-use technologies and minimal human interfaces allow for 2-3 operators to manufacture HIG product in ~30 hours. The overall platform is designed to minimize footprint, materials and time to support a distributed model of on-demand manufacturing. Verification of the operations will be completed in 2020 with the goal of initiating clinical safety testing 2020/2021.

549

KNOWLEDGE, ATTITUDES, PRACTICES, AND BELIEFS REGARDING PRENATAL ALCOHOL CONSUMPTION AMONG WOMEN IN LEYTE, THE PHILIPPINES

Alice Huang[1], Matthew Neale[1], Marianne Joy Sagliba[2], Amabelle Joy Moreno[2], Maria Paz Urbina[2], Mario Jiz[3], Jennifer Friedman[1]

[1]*The Warren Alpert Medical School of Brown University, Providence, RI, United States,* [2]*Research Institute for Tropical Medicine - Palo Satellite Laboratory, Palo, Leyte, Philippines,* [3]*Research Institute for Tropical Medicine, Muntinlupa, Metro Manila, Philippines*

Fetal alcohol spectrum disorder (FASD) encompasses varied emotional, behavioral, cognitive, and congenital abnormalities in individuals whose mothers consumed alcohol during pregnancy. In resource-limited settings, women may be at higher risk of having babies with FASD due to lack of systematic preventive education and consumption of unregulated brews. This is a major unaddressed public health issue in Leyte, The Philippines, where tuba, a locally made palm wine, is consumed. In this study, 100 postpartum women, established subjects in a longitudinal birth cohort in Leyte, were administered surveys in the local language of Waray via interview at their scheduled study visits in June-Sep. 2019. The survey examined participants' knowledge, attitudes, practices, and beliefs surrounding healthy pregnancies and their prenatal alcohol use. Chi-square and Fisher's exact tests were used to analyze bivariate relationships. 75% of subjects answered yes to drinking tuba and/or other alcoholic beverages during pregnancy. There were significant associations between reported prenatal tuba consumption and the false belief that tuba contains no alcohol (61% vs. 14% prenatal consumption respectively, chi-square=6.41, p=0.011) and significant associations between reported prenatal tuba consumption and the false belief that tuba has health benefits (45% vs. 30% prenatal consumption respectively, Fisher's exact p<0.05). Those who indicated that tuba has health benefits gave varied reasons, including tuba being nutritious and increasing lactation. Fifteen percent of subjects reported having fed their infants tuba for reasons such as to deworm or soothe them. Almost all surveyed (98%) indicated that they would cut back on drinking alcoholic beverages if they were told that this negatively impacted them and their unborn child. Overall, misinformation about tuba appears to play a role in informing the decisions of pregnant women in Leyte to drink tuba. Based on our results, educating women of reproductive age in Leyte regarding prenatal tuba use would likely lead to a reduction in tuba use among this population and FASD risk among their children.

550

FIELD EVALUATION OF MICROCHIP-BASED POINT-OF-CARE DEVICE 'GAZELLE' FOR DIAGNOSIS OF HAEMOGLOBIN DISORDERS IN INDIA

Shweta Srivas[1], Rajat Kumar[1], Anil Kumar Verma[1], Priyaleela Thota[2], Anne Rocheleau[3], Praveen K. Bharti[1], Tyler Witte[2], RamSwaroop Uikey[1], Anil Gwal[1], Umut Gurkan[4], **RajaSubramaniam S**[1]

[1]*ICMR - National Institute of Research in Tribal Health, Jabalpur, India,* [2]*Hemex Health, Portland, OR, United States,* [3]*Hemex Health, portland, OR, United States,* [4]*Case Western Reserve University, Cleveland, OH, United States*

Sickle Cell disease (SCD) is prevalent in the tropical regions of the world where malaria occurs. Hemoglobin (Hb) S is highly prevalent in sub-Saharan Africa, Mediterranean region, India and southeast Asia. India, Nigeria and Democratic Republic of Congo contribute about 57% of sickle cell anemia newborns globally. Generally, screening for HbS is performed using field tests such as solubility and sickling slide test followed by confirmation by High-performance liquid chromatography (HPLC) or hemoglobin electrophoresis. Here, we describe the performance of a microchip based cellulose acetate electrophoresis test "Gazelle™". The study was conducted in the tribal-dominated Indian states Madhya Pradesh and Chhattisgarh; HbS prevalence in this region varies from 3% to 35%. Gazelle™ is a rapid (<10 minutes), battery powered and easy-to-use test that uses only a finger-prick volume of blood and can be done even by minimally trained personnel. The device or the cartridges do not need any cold chain as well. Blood samples were collected from 1,050 patients who were enrolled in the study in a high prevalence setting (ICMR-NIRTH, India). Each sample was tested with Gazelle™, and the results were compared to electrophoresis and HPLC. Gazelle yielded a high accuracy (99.0%) compared to standard laboratory tests. Gazelle™ demonstrated high sensitivity and specificity for identifying SCD (HbSS) and β-Thalassemia, and sickle cell trait (HbAS). Gazelle showed 100% sensitivity comparing disease vs. trait and disease vs. normal. Specificity was 98.9% and 99.5% when comparing disease vs. trait and trait vs. normal, respectively. Specificity was 99.8% when comparing disease vs. normal and sensitivity was 99.3% when comparing trait vs. normal. Microchip electrophoresis technology offers a low-cost (~$2 per test), rapid, and accurate method for detecting hemoglobin disorders such as SCD. Gazelle can be a potential clinical tool for the rapid diagnosis of SCD and other hemoglobin disorders in under resourced and developing countries with high sickle burden.

551

COUNSELING AND INFORMED CONSENT RELATED TO MINIMALLY INVASIVE TISSUE SAMPLING (MITS) TO DETERMINE CAUSE OF DEATH FOR STILLBIRTHS AND NEONATAL DEATHS IN PAKISTAN, INDIA, BANGLADESH, KENYA AND ETHIOPIA: A COMPARATIVE EVALUATION

Tia Paganelli[1], Anam Feroz[2], Milka Bunei[3], B. Eshetu[4], Shahana Parveen[5], Sayyeda Reza[2], Sarah Saleem[2], Chaitali Sanji[6], Elizabeth McClure[7], Robert Goldenberg[8]

[1]*RTI International, Seattle, WA, United States,* [2]*The Aga Khan University, Karachi, Pakistan,* [3]*Influenza Program, Nairobi, Kenya,* [4]*Jimma University, Jimma, Ethiopia,* [5]*International Centre for Diarrhoeal Diseases Research, Bangladesh, Dhaka, Bangladesh,* [6]*Bapuji Hospital, Davangere, India,* [7]*RTI International, Research Triangle Park, NC, United States,* [8]*Columbia University, New York, NY, United States*

Globally, more than 5 million stillbirths and neonatal deaths occur annually. For many, the cause of death (CoD) is unknown. Minimally invasive tissue sampling (MITS) has been increasingly used in postmortem examinations for ascertaining the CoD in stillbirths and neonates. Our study compared the counseling and consent methods used in MITS projects in five countries in Africa and south Asia. Key informant interviews were conducted to describe the characteristics and backgrounds of counselors, the environment and timing of consent and perceived facilitators and barriers encountered during the consent process. Counselors at all sites had backgrounds in social science, psychology and counseling or clinical expertise in obstetrics/gynecology or pediatrics. All counsellors received training about techniques for building rapport and offering emotional support to families; training duration and methods differed across sites. Counselling environments varied significantly; some sites allocated a separate room, others counselled families at the bedside or nursing stations. All counsellors had a central role in explaining the MITS procedure to families in their local languages. Most sites did not use visual aids during the process, relying solely on verbal descriptions. In most sites, parents were approached within one hour of death. The time needed for decision making by families varied from a few minutes to 24 hours. In most sites, extended family took part in the decision making. Counsellors emphasized that MITS was less invasive compared to full autopsy. Because many parents wanted burial as soon as possible, counsellors ensured that MITS would be conducted promptly after receiving consent. Barriers to consent included decreased comprehension of information due to the emotional and psychological impact of grief. Moreover, having more family members engaged in decision-making increased the complexity of counselling and achieving consensus to consent for the procedure. While each site adapted their approach to fit the context, consistencies and similarities across sites were observed.

552

COMMUNITY HEALTH WORKERS IN DOMINICAN REPUBLIC BATEYES: THE INFLUENCE OF PROGRAMMATIC AND CONTEXTUAL FACTORS

Shanelle D'Alessio[1], Odalis Ruiz[2], Magalie Joseph[2], David Hill[1]

[1]*Frank H. Netter School of Medicine at Quinnipiac University, North Haven, CT, United States,* [2]*Hospital El Buen Samaritano, La Romana, Dominican Republic*

Community Health Workers (CHWs) have been recognized by the World Health Organization as a potential avenue to increase global healthcare coverage. While there is robust evidence that CHWs can be effective in healthcare delivery, the factors that affect CHW performance are still unclear. In La Romana, Dominican Republic, a CHW program provides health services to hundreds of under-resourced batey communities. This study aimed to elucidate the factors that promote and limit the success of batey CHWs through a parallel, mixed-method approach. Individuals employed as CHWs (N=47) by a La Romana hospital were invited to complete a self-administered questionnaire. A convenience sample (n=20) completed a semi-structured interview. Survey items focused on factors identified by the WHO as potentially relevant to CHW program function. Of 40 CHWs who completed the questionnaire, CHW age, education level, work experience, batey size, and batey location varied greatly. 90% endorsed the statement that their work is always useful to their community. However, the majority also expressed a need for new skill trainings (88%), hospital support (83%), community recognition of their work (83%), higher pay (78%), medical supplies (73%), continued education (70%), and supervisory communication (68%). Only 38% reported they would benefit from an additional CHW in their batey. Overall, training administration, data collection, and community fit promoted the success of the CHW program in La Romana bateyes. However, CHWs perceived a need for improvement in multiple areas: workload, frequency and focus of trainings, supply provisions, integration into the healthcare system, and remuneration. Thematic analysis of interviews contextually broadens these findings. Final conclusions aim to inform development of CHW programs in bateyes and similar contexts.

553

ROUTINE IMMUNIZATION STATUS OF CHILDREN UNDER FIVE LIVING IN 11 HIGH MALARIA TRANSMISSION COMMUNITIES IN MALABO DISTRICT, BIOKO ISLAND

Esther Eburi Losoha[1], Maria Silvia Lopez Mikue[2], Vicente Urbano Nsue Ndong[2], Antonio Enrique Ngua Roca[1], Gabriel Mba[1], Ines Toichoa[1], Marcos Mbulito[1], Jordan Smith[1], Ali Hamad[3], Kamaka Ramadhani[3], Jongo Said[3], Wonder Philip Phiri[1], Guillermo Garcia[1], Claudia Daubenberger[4], Sonja Merten[4]

[1]*Medical Care Development International, Malabo, Equatorial Guinea,* [2]*Minitry of Health and Social Welfare, Malabo, Equatorial Guinea,* [3]*Ifakara Health Institute, Bagamoyo, United Republic of Tanzania,* [4]*Swiss Tropical and Public Health Institute, Basel, Switzerland*

According to the United Nation International Child Emergency Funds (UNICEF) between 2009 and 2018, the coverage of routine immunization has been declining at an estimated rate of 10%-20% in Equatorial Guinea. Although the national Expanded Program of Immunization (EPI) with UNICEF has made progress in the elimination of Polio in 2019, there is still a lack of quality research data. The purpose of this study is to improve the quality of the estimated data of routine coverage, which then will help to identify the risk factors that contribute to children not vaccinated at the community level. The objective is to describe socio-demographic status. Also, to determine the actual coverage rate of routine immunization among participants using both estimated national census data and Bioko Island Malaria Elimination Project (BIMEP) census data. Moreover, to determine the associated factors for routine immunization rate among participants of a pilot study (EG-RESPAR) that aims to optimize recruitment, screening procedures to create a registry of 3000 potential research participants for future Phase III malaria vaccine clinical trial in Malabo district, Bioko Island. A descriptive cross-sectional assessment of routine immunization data of children less than five years old that reside in both urban and rural high malaria transmission communities. Caregivers will provide immunization history of children and data verification will be done by vaccination card or by a recall, including vaccination health clinics used for their children. Besides, the proportion of children under the age of five who are fully immunized will be evaluated according to the national routine vaccination schedule. These results will contribute to the knowledge gap and will also help to identify potential factors that lead to low immunization coverage among children under the age of five in this study setting. The study will provide information for decision-makers to identify policy gaps and develop public health strategies to improve immunization adoption among those most vulnerable to vaccine-preventable diseases.

555

HEALTH SERVICES UTILIZATION BY INDIVIDUALS WITH ACUTE INFECTIOUS SYNDROMES IN THE HIGHLANDS OF GUATEMALA, 2020

Laura Maria Grajeda[1], Celia Cordón-Rosales[1], Brooke M. Ramay[1], María Renee López[1], Ana Gómez[2], César Sánchez[2], John P. McCracken[1], Douglas R. Call[3]

[1]*Universidad del Valle de Guatemala, Guatemala, Guatemala,* [2]*Ministerio de Salud Pública y Asistencia Social, Guatemala, Guatemala,* [3]*Washington State University, Pullman, WA, United States*

The Collaborative Integrated Surveillance (VICo) program focuses on acute infections at a Guatemalan regional public hospital. As part of VICo, we sought to estimate the health utilization patterns within this hospital catchment area. A three-stage cluster sampling scheme was used to randomly select one member of each participating household. We estimated the prevalence of self-reported acute febrile illness, influenza-like illness and diarrheic syndrome with onset during the previous month. Prevalence estimates were adjusted for selection and response weights. We surveyed 418 individuals for acute infectious (76% female, median age of 31 years, interquartile range of 15 to 51 years). Reported syndromes included acute febrile illnesses (9%, 95%CI: 6-12, n=40), influenza-like illness (12%, 95%CI: 9-15, n=57), and diarrhea (14%, 95%CI: 10-18, n=58). Among 90 (86%) of these cases, individuals sought assistance at a public healthcare facility in 24% (95%CI: 6-42, n=7), 26% (95%CI: 14-39, n=12) and 11% (95%CI: 3-20, n=7) of febrile, respiratory and diarrheic cases, respectively. Of these, two respiratory and one diarrhea cases sought care at the surveillance hospital, while all others visited public clinics. A higher proportion, 39% (IC95%: 18-60, n=7), 36% (IC95%: 18-54, n=12), and 60% (IC95%: 45-75, n=7), respectively, did not seek care outside the household. The rest sought care at private facilities. These findings indicate that hospital surveillance could only capture three out of 90 cases occurring in the community. Extending surveillance to public clinics will increase coverage to approximately 25% of cases. The inability to seek appropriate care outside the household could impact morbidity and mortality from these diseases. The Global Action Plan for the Control of Pneumonia and Diarrhea recognizes case management in health facilities as a key strategy to achieve its goals. These reports are similar to findings in 2009 in the same area, except for a previously lower estimated prevalence of diarrhea, 8% (IC95%: 7-9). A major limitation of our design is that we used a recall period of 30 days whereas bias can be expected after 7 days.

556

INTEGRATED MENTORING AND SUPERVISION VISITS IMPROVE MALARIA SERVICE DELIVERY IN HEALTH FACILITIES IN DELTA STATE, NIGERIA

Chinedu Chukwu[1], Serah Nweke[1], Victoria Erinle[1], Melis Esi[1], Isaac Adejo[1], Mariah Boyd-Boffa[2], Stephanie Xueref[3], Tom Hall[3], Sonachi Ezeiru[4], Frank Oronsaye[4], Diwe Ekweremadu[4], Temitope Ipinmoye[4], Bala Mohammed Audu[5], Perpetua Uhomoibhi[5], Festus Okoh[5], Ibrahim Maikore[5], Nnenna Ogbulafor[5], Emmanuel Shekarau[5], Beauty Okologo[5], Bolakale Kawu Issa[5]

[1]*Management Sciences for Health (MSH), Abuja, Nigeria,* [2]*Management Sciences for Health (MSH), Medford, MA, United States,* [3]*Management Sciences for Health (MSH), Arlington, VA, United States,* [4]*Catholic Relief Services (CRS), Abuja, Nigeria,* [5]*National Malaria Elimination Program (NMEP), Abuja, Nigeria*

Training and supervision of health workers are two key elements related to quality of malaria service delivery. Prior to 2018, health workers in Delta State, Nigeria were not routinely supervised and the last training they had received on malaria was in 2013. In January 2018, Management Sciences for Health, in collaboration with the National Malaria Elimination Program and Catholic Relief Services, using resources provided by the Global Fund Malaria Grant (2018-2020), established a training program coupled with an integrated mentoring and supervision visit (iMSV) approach to improve malaria health service delivery. Both the training and iMSV focused on malaria case management, surveillance monitoring and evaluation, and supply chain management, with an emphasis on improving the quality of service delivery through adherence to guidelines, and rigorous documentation and reporting. Between January 2018 and December 2019, 3,857 health workers were trained, while iMSV reached 379 of 400 public health facilities and included six quarterly, four biannual and nine monthly visits across 21 local government areas. The iMSV team focus was to reinforce training objectives and mentor health workers, which included providing feedback and progress reports based on previous visits. Performance improvement was monitored via three, malaria-service indicators including proportion of confirmed malaria cases treated with an artemisinin-based combination therapy (ACT); proportion of clinically diagnosed malaria cases; and, proportion of pregnant women attending antenatal clinic who received at least one dose of intermittent preventive treatment (IPTp1). The data show that the proportion of confirmed malaria cases treated with an ACT improved from 73% in quarter 1 (Q1) 2018 to 99% in quarter 4 (Q4) 2019. The proportion of clinically diagnosed malaria cases declined from 76% in Q1 2018 to 11% in Q4 2019. The proportion of pregnant women receiving IPTp1 improved from 14% in Q1 2018 to 60% in Q4 2019. The results indicate that training reinforced with regular iMSV may contribute to improving key indicators related to quality malaria service delivery.

557

LEVERAGING LEADERSHIP AND GOVERNANCE STRUCTURES TO STRENGTHEN SUPPLY CHAIN DATA MANAGEMENT SYSTEMS AND ACCOUNTABILITY THROUGH BI-MONTHLY TRIANGULATION OF LOGISTICS MANAGEMENT INFORMATION AND HEALTH MANAGEMENT INFORMATION DATA SETS: A CASE STUDY OF 1213 PRIMARY HEALTHCARE CENTERS IN KANO STATE, NIGERIA

Isaac Adejo[1], Melis Esi[1], Zuman Tangshak[1], Stephanie Xueref[2], Tom Hall[2], Chukwuemeka Akam[1], Olumide Elegbe[1], Beauty Okologo[3], Kenji Goyit[3], Sam Abutu[3], Victor Adebayo[4], Ezinne Onuba[4], Stanley Chidove[5]

[1]*Management Sciences for Health (MSH), Abuja, Nigeria,* [2]*Management Sciences for Health (MSH), Arlington, VA, United States,* [3]*National Malaria Elimination Program (NMEP), Abuja, Nigeria,* [4]*Catholic Relief Services (CRS), Abuja, Nigeria,* [5]*The Global Fund, Geneva, Switzerland*

Availability of malaria health products (MHP) is a cornerstone of the National Malaria Elimination Program (NMEP) effort to end malaria in Nigeria; however, accountability of these MHP has been a consistent issue. Specifically, the country has experienced wide and consistent variance between consumption and service data (> ± 10%) at the health facility (HF) level as captured by the National Health Logistics Information System (NHLMIS) and the Nigeria Health Management Information System (NHMIS) respectively. Contributing factors in Kano State performance variance (235%) include inadequate strategic direction and decision-making provided by supply chain leadership (SCL), and weak governance structures, which foster poor transparency and oversight practices, leading to low accountability. In June 2019, Management Sciences for Health, in collaboration with the NMEP and Catholic Relief Services, under the Global Fund Malaria Grant, used an Excel-based, bimonthly-data-triangulation-diagnostic-and-intervention tool to improve supply chain data management systems and accountability in 1213 HFs in Kano State. Specifically, the tool links NHLMIS and NHMIS data allowing SCL, at different levels, to assess if consumption of MHP correlates with services provided to patients in a given HF. Use of the tool includes dissemination, technical assistance on the tool's data analytics, and leveraging of findings to SCLs of the State Ministry of Health including the Supply Chain Technical Working Group in order to guide targeted supervision and on-the-job training for poor performing facilities. Results show that the proportion of HFs falling within the allowable ± 10% range of variance increased from 7% to 28% for rapid diagnostic tests, 2% to 22% for artemisinin-based combination treatments, and 3% to 18% for long-lasting insecticidal nets between March-April 2019 and January-February 2020; and with an overall performance variance from 235% to 111%. These findings indicate that empowering decision-makers and supervisors to use data-driven tools contributes to improved management of MHP in HFs.

558

IMPACT OF A DATA QUALITY IMPROVEMENT TOOL IN ENHANCING SUPPLY CHAIN DATA QUALITY AT HEALTH FACILITIES: A CASE STUDY OF 300 HEALTH FACILITIES IN ADAMAWA, KADUNA AND KWARA STATES, NIGERIA

Melis Esi[1], Isaac Adejo[1], Stephanie Xueref[2], Tom Hall[2], Abdullahi Mohammed[1], Olumide Elegbe[1], Beauty Okologo[3], Kenji Goyit[3], Victor Adebayo[4], Peter Biaghere[5], Stanley Chidove[6]

[1]*Management Sciences for Health (MSH), Abuja, Nigeria,* [2]*Management Sciences for Health (MSH), Arlington, VA, United States,* [3]*National Malaria Elimination Program (NMEP), Abuja, Nigeria,* [4]*Catholic Relief Services (MSH), Abuja, Nigeria,* [5]*Catholic Relief Services (CRS), Abuja, Nigeria,* [6]*The Global Fund, Geneva, Switzerland*

Poor quality of data in the Bimonthly Facility Stock Report (BFSR) has been of great concern to the malaria health products supply chain management partners in Nigeria. To address this issue, Management Sciences for Health, with funding from the Global Fund Malaria Grant (2018-20), and in collaboration with the Catholic Relief Services and the National Malaria Elimination Program, developed a BFSR improvement tool (BFSRIT), to be used at the state level by the Logistics Management Coordinating Units (LMCUs), to rate the BFSR quality (BQ) at each health facility (HF), and to assess and monitor their performance over time. The purpose of this study is to assess the impact of the newly developed BFSRIT in enhancing the BQ generated by a total of 300 HFs randomly selected across all facility levels in three states - Adamawa, Kaduna and Kwara. The tool provides a framework to assess and rate the accuracy of the nine data elements on the BFSR. A cross-sectional data analysis method, using various sources (such as Proof of Deliveries) of information to determine quality of the data reported in the BFSR, was used to establish a grade (1= accurate or 0= not accurate). The grades are summed to establish a quality score by HF and an average score by state. We conducted a baseline analysis of the May-June 2019 reports, which showed that at the sampled facilities, the BQ was 51%, 49% and 53% for Adamawa, Kaduna and Kwara respectively. Technical assistance was provided to the states' LMCUs to review and monitor the quality of the data in the BFSRs from the sampled facilities using the BFSRIT. The data quality assessment conducted by the LMCUs provides access to granular performance data by HF, which was used to

inform targeted capacity building to poor performing HFs. By the end of three reporting cycles (ending February 2020), analysis of BFSRs from the HFs revealed that the BQ improved across the sampled HFs, in each of the states to 52%, 55% and 76% in Adamawa, 53%, 68% and 84% in Kaduna and 84%, 84% and 95% in Kwara. The results demonstrate that the BFSRIT provides evidence to guide targeted capacity building support to HFs, which resulted in an improvement of data quality.

559

PUBLIC AWARENESS ABOUT ANTIBIOTIC AND ITS USE: A PROSPECTIVE STUDY AMONG KAVRE AND KATHMANDU POPULATION OF NEPAL

Krista Vaidya[1], Jason R. Andrews[2], Dipesh Tamrakar[1], Rajeev Shrestha[1], Denise Garrett[3], Isaac I. Bogoch[4], Kristen Aiemjoy[2]

[1]*Dhulikhel Hospital, Dhulikhel, Nepal,* [2]*Stanford University, Stanford, CA, United States,* [3]*Sabin Vaccine Institute, Washington, DC, United States,* [4]*University Health Network-Toronto General Hospital, Toronto, ON, Canada*

Antibiotic resistance is the global health crisis. Easy availability, unnecessary & inappropriate use of antibiotics drives to antibiotic resistance. Studies have shown that lack of antibiotic awareness is associated with antibiotic resistance. In Nepal, few studies were done to observe antibiotic awareness. This study aims to explore antibiotic awareness among the general population of urban & peri-urban Nepal. We conducted a random population-based survey in five urban & peri-urban municipalities. Using geospatial sampling, we randomly selected clusters, enumerated all households living within the cluster & drew a random age-stratified sample of an index person between 0 & 25 years. We interviewed the head of household or index person &gt 18 years about antibiotic awareness & its use using a structured questionnaire & collected information about education, occupation & income. We used mixed effects models with a random effect for sampling cluster to evaluate if variation in antibiotic awareness was determined by district, education or income. We enrolled 904 households from January 2019 to February 2020. The respondents were mother (320/904), father (131/904) or grandparent (27/904) of the index person or the index person themselves (267/904). Most of the participants were aware of the term antibiotic (86.2%; 779/904). Individuals residing in a peri-urban district were .53 times less likely to have heard the term antibiotic compared to individuals residing in an urban district (p=0.008). Households in the wealthiest income range were 3.7 times more likely to have heard the term antibiotic compared to households in the lowest income range (p=0.02). Nearly a quarter of participants indicated that antibiotics should be taken for a cold or a flu (21%, 191/904). Only 10.1 % (90/893) could name an antibiotic. Most of the participants were aware of the term antibiotic however, few could provide the name. Antibiotic awareness was more common in urban areas & among wealthier households. These findings can be useful in interpreting antibiotic resistance results & focus female, peri-urban & low-income group in future interventions.

560

EXPERIENCES OF THE DECEASED CHILDREN FAMILIES AFTER CONSENT TO MINIMALLY INVASIVE TISSUE SAMPLING (MITS) IN MOZAMBIQUE: A QUALITATIVE STUDY

Amílcar Magaço[1], Yury Macete Macete[1], Nelson Escritorio[1], Raquel Mucore[1], Antonio Calia[1], Quique Bassat[2], Inacio Mandomando[1], Maria Maixenchs[2], Khatia Munguambe[1]

[1]*Manhica Health Research Center, Manhica, Mozambique,* [2]*ISGlobal, Hospital Clínic - Universitat de Barcelona, Barcelona, Spain*

Mozambique faces the paradox of a high child mortality rate versus low vital registration coverage. The Countrywide Mortality Surveillance for Action (COMSA) is designed to strengthen vital registration systems while investigating causes of death (CoD) in children under 5 through verbal autopsies (VA). In Quelimane (Central Mozambique), Minimally Invasive Tissue Sampling (MITS) is being implemented since 2019 to complement VA for CoD investigation. During this implementation, a rapid assessment was conducted to assess MITS acceptability and understand the experiences of deceased children families who consented to MITS. Data were collected through semi-structured interviews (SSI) with deceased children family members and systematic field observations of mortality surveillance activities and local post-mortem events. The SSI were transcribed and coded using NVIVO12, later the coded SSI were analyzed in triangulation with the observations data. A total of 50 SSI and 20 observations were conducted. High consent rates were consistently observed. The limited available information about MITS procedures leading to a lack of understanding of the procedures being performed contributed to the dissatisfaction of some families. In addition, the time required to perform MITS and deliver the results to the families was described as a factor that increased anxiety among family members and thus may compromise the long-term acceptability of MITS. Tension between the core family members who consented and the members of the wider family was noticed due to the latter members' lack of opportunity to have a say during the consent process. This was exacerbated by the displeasure with the level of conservation or integrity of the body when released after MITS performance. Family experiences after consent were influenced by several operational factors related with MITS procedures and contextual factors associated with decision-making to perform MITS, mostly informed by the cultural values. This study suggests that despite an apparent high acceptance rate, families require continuous information and support after the consent and the execution of MITS.

561

DO THE RIGHT THING: NEGLECTED DISEASES AS A MODEL FOR EFFICIENT DRUG DEVELOPMENT

Eric Ipyn Nebie[1], Jurg Utzinger[2], Christian Burri[2]

[1]*Centre de Recherche en Santé de Nouna CRSN, Nouna, Burkina Faso,* [2]*Swiss TPH University of Basel, Basel, Switzerland*

Drug development is a costly, and complex process. The development of a single new drug costs over US$ 2.6 billion in the pharmaceutical industry. Attrition rates and trials length are cited as the main drivers of R&D efficiency decline. A quarter of R&D inefficiency is related to trial processes, study complexity, quality approach and management. Similarly, the prices of new drugs exponentially increased up to US$ 350,000 for a single treatment in a Precision Medicine approach in the North. Such high drug prices overburden the global health systems and pose a major challenge of access equity. For the development of drugs for use in low resource countries and against poverty-related diseases, virtual drug developing organizations, Product Development Partnerships (PDPs) emerged. PDPs are not-for-profit, small think tanks striving to use cost-cutting strategies (lean processes, networking) to develop drugs for a quarter or less of the industry estimated costs. Our aim is to assess cost, process and resource efficiency in industry, PDPs and academia through a comparison of processes, quality approaches management and expenditures; in turn we will identify potential shortcomings in PDPs, academia and industry. An initial literature review of clinical trials efficiency assessment methods will allow us to elaborate a standardized efficiency assessment methodology. Then a sequential exploratory mixed method design will be used. The qualitative component will analyze the trial organization, processes, quality approaches, Good Clinical Practices, regulatory burden and management through direct observations and semi-structured interviews conducted during immersions in various research settings. Thereafter, an online quantitative questionnaire will complement the qualitative component. We will present the approach to this endeavor, the preliminary results of the literature review and of the first immersions at Sub-Saharan excellence centers in clinical research including cost-saving factors on trial management procedures, participant's recruitment and retention strategies comparing in industry and PDPs models.

562

IDENTIFYING AND UTILIZING COMMUNITY CHAMPIONS AND RESOURCE PERSONS TO INCREASE MITS ACCEPTANCE IN BANGLADESH

Faruqe Hussain[1], Emily Gurley[2], John Blevins[3], Ahoua Kone[3], Abdullah Al Masud[1], Abdus Suban Mulla[1], Abu Uzayer[1], Afroz Zahan[1], Aziz Ahamed[1], Shikha Datta Ggupta[1], Suruj Ali[1], Farhana Hasnat Khan[1], Safiur Rahman[1], Md. Salim Reza[1], Md. Al Mamun[1], Md. Mahadi Hassan[1], Sazzad Hossain[1], Tonmoy Sarkar[1], Dalia Yasmin[1], Mamunur Rashid[1], Ahmed Shahriar[1], Palash Mutsuddi[1], Farzana Islam[1], Shams El Arifeen[1], Shahana Parveen[1]

[1]*icddr,b, Dhaka, Bangladesh,* [2]*Johns Hopkins Bloomberg School of Public Health, Maryland, MD, United States,* [3]*Emory University, Atlanta, GA, United States*

The Child Health and Mortality Prevention Surveillance (CHAMPS) program is implementing postmortem minimally invasive tissue sampling (MITS) procedures to identify the aetiology of under 5 child deaths in Baliakandi sub district, Bangladesh. The MITS procedure is new in Bangladesh and evokes socio-cultural, emotional and religious concerns that influence acceptance. We aimed to identify community residents to engage and address these concerns that can support to MITS consent. From March 2019 to February 2020, we identified 117 community recommended local residents as community champions and resource persons who had 1) prior experience with the MITS procedure and 2) participation in sessions reporting on the cause of death based on data that could only be gathered through MITS. To facilitate MITS consent in the facilities and community, we oriented the champions and resource persons to voluntarily share their personal experience and to clarify the MITS process whenever they were referred and connected to the family of a deceased child were approached for MITS consent to support their decision making. Our team approached families at a medical facility when a child died there and in the community when a child died outside a medical facility. These champions and resource persons provided support during community entry and responded to religious queries raised by the deceased families. In facilities, during the MITS informed consent approach, 46 families were proposed to consult with community champions but only two families consulted and consented to MITS. Resource persons supported the team that approached families of deceased children in the community for MITS consent; 4 out of 13 families (31%) consented. However, their voluntary involvement and support during the consent approach in the community was limited because of their livelihood and social concerns. Community champions are useful for approaching in the facility and resource persons in the community in facilitating MITS acceptance and consent. Recruiting more community champions and resource persons could help ensure they can support in approaching families for MITS consent.

563

FACTORS ASSOCIATED TO TIME TO PREGNANCY: A PROSPECTIVE PRECONCEPTIONAL STUDY IN BENIN, WEST AFRICA

Emmanuel Koffi Yovo[1], Manfred Accrombessi[2], Gino AGBOTA[3], Achille MASSOUGBODJI[1], Valérie BRIAND[4]

[1]*Institut de Recherche Clinique du Bénin, Abomey-Calavi, Benin,* [2]*Faculty of Infectious and Tropical Diseases, Disease Control Department, London School of Hygiene & Tropical Medicine, London, United Kingdom,* [3]*Université de Paris, UMR261-MERIT, IRD, Paris, France,* [4]*MERIT, IRD, Université Paris Descartes, Sorbonne Paris Cité, France*

There are few data on the burden and risk factors of hypo/infecundity in sub-Saharan Africa. Therefore, studying factors associated with women's fecundity at population-level is of obvious usefulness. Time to pregnancy (TTP) is the number of month elapse between the start and the end of the period of unprotected intercourse to obtain a pregnancy. We use data from a preconceptional cohort study to investigate the association between a wide range of epidemiological and biological factors and TTP in Southern Benin. Data were extracted from RECIPAL study (2014 to 2017) for which women of reproductive age willing to become pregnant were followed up at community-level. The last menstrual period was reported and a urinary pregnancy test was performed monthly. Associations between the investigated factors and TTP were assessed using Cox regression analyses. Among 1073 women whose data was finally used for analysis, 411 (38.3%) of them get pregnant during follow-up, 359 (33.5%) were still not pregnant at the end of the follow-up and 303(28.2%) had an incomplete follow-up. The mean (SD) duration of follow-up was 12 (6.3) months and the 25th, 50th, 75th and 95th percentiles were 6, 10, 16 and 23 months respectively. The median TTP for the overall cohort was 13 months and after restricting to the 411 women who get pregnant during follow-up, it was 4 months. The cumulative probability of conception at 3; 6; 12 and 18 months of follow-up were 0.42; 0.65; 0.91 and 0.96 respectively for the 411 women. With the Cox adjusted model, 27% and 59% reduction in fecundability was observed in women aged 21 - 32 years (FOR: 0.73, 95 CI: 0.54 - 0.98) and > 32 years (FOR: 0.41, 95 CI: 0.27 - 0.61) respectively, when compared to women aged < 21 years. Overweight and obesity are associated with a reduction in fecundability of 23% (FOR: 0.77, 95 CI: 0.59 - 1.00) and 31% (FOR: 0.69, 95 CI: 0.48 - 1.01) respectively, when compared to optimal weight women. Based on preconceptional data, we showed that the median TTP among women becoming pregnant was 4 months in southern Benin. Women's age and nutritional status influenced fecundity.

564

SUSTAINING EBOLA MANAGEMENT BY PREDICTIVE MODELING

Aliou Bouba[1], Vincent Appiah[2], Martin Eichner[3], Kristan Alexander Schneider[4]

[1]*African Institute for Mathematical Sciences of Cameroon (AIMS-CAMEROON)), Limbe, Cameroon,* [2]*West African Centre for Cell Biology of Infectious Pathogens, Accra, Ghana,* [3]*University of Tübingen, Tübingen, Germany,* [4]*Hochschule Mittweida University of applied sciences, Mittweida, Germany*

The Ebola epidemic of 2014-2016 in three West African countries and the consecutive outbreak in Uganda and DRC challenged global health-emergency management. During the initial phase of an epidemic, when valid epidemiological data is yet unavailable, it is indispensable to sustain crisis management by predictive models to evaluate the impact of potential control interventions. During the recent Ebola outbreaks in West and Central Africa, the impact of various control interventions (e.g., case isolation, quarantine of exposed, contact tracing, safe burial ceremonies in traditional tribes) has been assessed individually by a number of predictive models. However, these interventions have not been studied in combination. Here, the effect of the interaction of various interventions is studied in combination with a compartmental-type (SEIR) predictive model. The model describes the population of susceptible, latent, early- and (hospitalized and non-hospitalized) late-infectious, recovered and dead individuals under a combination of intervention strategies. We verbally describe and illustrate the model and present simulation results illustrating the effect of interventions obtained with the easy-to-use software Berkeley Madonna. We found that the epidemic is controllable, thereby effectively reducing disease burden, by effective contact tracing, case isolation, and safe burial ceremonies reduce disease burden. The model is extendable to account for the interaction of human and natural Ebola reservoirs (e.g., bats) leading to new disease outbreaks.

565

OVERCOMING BARRIERS FOR TIMELY NOTIFICATION OF COMMUNITY DEATHS IN THE CONTEXT OF A MORTALITY SURVEILLANCE PROGRAMME IN SOUTHERN MOZAMBIQUE

Rui A. Guilaze[1], Maria Maixenchs[2], Constantino Cumbane[1], Inácio Mandomando[3], Ariel Nhacolo[1], Quique Bassat[4], Khátia Munguambe[5]

[1]*Manhica Health Research Centre, Manhiça-Maputo, Mozambique,* [2]*IsGlobal, Hospital Clinic - Universitat Barcelona, Barcelona, Spain,* [3]*Instituto Nacional de Saúde, Ministério da Saúde, Manhiça-Maputo, Mozambique,* [4]*Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain,* [5]*Eduardo Mondlane University, Faculty of Medicine, Maputo, Mozambique*

Identifying causes of death (CoD) is crucial to reducing under-5 mortality, mostly caused by preventable diseases. Child Health Mortality Prevention Surveillance (CHAMPS) is implementing Minimally Invasive Tissue Sampling (MITS) to track the CoD in children under-five. MITS must be conducted within 24 hours of the death to ensure accuracy of results; this requires an efficient death notification system. We conducted a qualitative rapid assessment to describe CHAMPS' process of notification of deaths occurring outside the health facility (*community deaths, CD*) and barriers encountered, in Manhiça, Mozambique. Data were collected between February and March, 2020 through 11 semi-structured interviews, 1 focus group discussion (FGD) and 1 informal conversation, involving 20 participants (parents and caregivers of deceased children, and community leaders). CD notifications are centralized by the *Secretários de Bairro* (neighbourhood chiefs, NC). When a death occurs, the family informs the nearest community leader, usually the *head of 10 houses*, who informs the *chief of 60 houses*, who conveys the information to the NC, who in turn formally notifies the surveillance team through the CHAMPS call centre. Low-level community leaders are discouraged from reporting deaths to third parties without the NC's authorization, although during the FGDs there were no objections. Lack of awareness of the call centre's phone numbers, operators' occasional unavailability, and failure to return the NC missed calls were the barriers faced by participants. Other barriers mentioned were family members' forgetfulness of reporting a death due to their focus on funeral arrangements, difficulty to reach out NCs living far from where the death occurred and/or their absence at the time of notification, and lack of cell phone airtime. Key recommendations for improving death notifications included a decentralized death notification system, involving different community members without jeopardizing community structures of power relations, community sensitization on the importance of reporting deaths, and using a toll-free call centre service.

566

ADOPTION OF A NATIONAL SYSTEM FOR ANTIMICROBIAL SUSCEPTIBILITY TESTING FOR ANTIMICROBIAL RESISTANCE SURVEILLANCE IN NIGERIA

Abiodun J. Egwuenu[1], Joshua Obasanya[1], Tim Eckmanns[2], Biodun Ogunniyi[1], Sara Tomcyzk[1], Chikwe Ihekweazu[1]

[1]*Nigerian Centre for Disease Control, Abuja, Nigeria,* [2]*Robert Koch Institut, Berlin, Germany*

An integral part of AMR surveillance is antimicrobial susceptibility testing (AST), which depends on using set breakpoints. Interpretation of "resistance" levels is critical to patient care and maintaining the efficacy of treatment regimens. The two most popular AST standards are the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Due to increasing global focus on AMR, selection of an AST methodology is becoming increasingly important. A consultative meeting for 35 national stakeholders was coordinated by Nigeria Centre for Disease Control in September 2018 to discuss the merits and demerits of switching from CLSI to EUCAST. Participants were clinical microbiologists, medical laboratory scientists, epidemiologists, policy makers and public health experts from ministerial bodies, laboratories, regulatory agencies, and development partners. Stakeholders were engaged via interactive discussion sessions and findings were summarized thematically. All the participants stated that they were more familiar with CLSI. However, majority did not have access to the most updated version of the guidelines due to the cost. Perceived benefits of EUCAST were that Francophone West African countries conducting surveillance for AMR use the guidelines, allowing for data comparism and its freely available nature. However, EUCAST does not provide disk diffusion breakpoints for *Neisseria meningitidis* and *Vibrio Cholerae*, two of eight pathogens prioritized for AMR surveillance in Nigeria. Stakeholders also noted that access to horse blood for media preparation and use of alternative methods such as E-test for pathogens for which breakpoints are unavailable, were challenges. However, updated CLSI documents must be purchased annually, which really affects timely access to up-to-date guidelines. The priority for stakeholders was to improve workforce capacity and ensure access to necessary equipment and consumables to enhance diagnostic capacity. Nigeria is open to adopting the EUCAST guidelines for AST. However, a sustainability plan needs to be developed.

567

IMPROVING ACCESS TO CARE AND COMMUNITY HEALTH IN HAITI WITH OPTIMIZED COMMUNITY HEALTH WORKER PLACEMENT

Clara Champagne[1], Jean-Patrick Alfred[2], Yves Gaston Deslouches[2], Marvin Ploetz[3], Andrew Sunil Rajkumar[3], Phoebe Meyer[4], Katherine Battle[5], Ewan Cameron[5], Giulia Perrone[6], Paul Auxila[7], Emilie Pothin[1]

[1]*SwissTPH/University of Basel, Basel, Switzerland,* [2]*Ministère de la santé publique et de la population, Port-au-Prince, Haiti,* [3]*World Bank, Washington, DC, United States,* [4]*Clinton Health Access Initiative, New York, NY, United States,* [5]*Malaria Atlas Project, Oxford Big Data Institute, Li Ka Shing Centre for Health Information and Discovery, University of Oxford, Oxford, United Kingdom,* [6]*Global Fund, Geneva, Switzerland,* [7]*Global Financing Facility, Port-au-Prince, Haiti*

Due to social and political vulnerabilities, as well as the number of natural disasters that Haiti had to face, the current health system cannot guarantee health service access and quality to the majority of the population. The deployment of polyvalent community health workers (CHWs), covering population in urban, rural and difficult-to-reach areas is a constitutive part of the person-centred primary care reinforcement initiated by the ministry of health and will accelerate efforts to reach universal health coverage. For its implementation, two essential elements need to be revisited: the organisation of the community health system as well as the package of services delivered. A methodology based on mathematical tools was developed to support the development of guidelines and inform the geographical deployment of Community Health Workers (CHWs) in Haiti. Fine-graded estimates of population and travel times were combined with integer programming optimisation methods to derive placement scenarios that account for population density, road networks and topography. In order to give guidance on important operational limitations, parameters included constraints on walking time and number of people allocated to each CHW, as well as proximity to existing health facilities. Several national-scale scenarios adapted to the Haitian context were compared, in order to inform the number and distribution of CHWs required to bridge the gap in access to health services. The results of the analysis advised the development of the National Strategic Community Health Plan by providing guidance on the expected number each CHW could serve and their catchment area. The planning tool developed to help target limited resources and optimize Haiti's revised community health system may prove useful in programming and costing community health plans in additional contexts.

568

REVEAL: OBSERVATIONS AND COVERAGE GAINS IN A GEOSPATIAL TOOL IN USE OVER SIX YEARS IN ZAMBIA

Anne Martin, Christina Riley, Benjamin Winters, Derek Pollard, Frazer Bwalya, Anna Winters

Akros, Lusaka, Zambia

The Reveal system, formerly mSpray, was one of the first tools to leverage geospatial data to provide real-time in-field navigation to guide health teams to target households and communities. Reveal has been in use in Zambia since 2014, in 5 provinces, for Indoor Residual Spraying (IRS), and Mass Drug Administration (MDA; for both malaria and schistosomiasis). We will present descriptive statistics for each Reveal implementation. Coverage is defined as the percentage of eligible units that received the service in question. Denominators are estimated from remotely sensed enumeration of satellite imagery and are partially verified as teams deliver services in the field. We will therefore present two coverage metrics derived from two denominators - the first is the known denominator after field verification and the second is an adjusted denominator that applies the verification rates to not visited structures. We will present each of these coverage metrics at all levels of the geographic hierarchy and will present various coverage deltas for areas that have been delivered the same service, using Reveal, for multiple years. We will assess and attempt to build a model for both the coverage and coverage deltas, examining potential cofactors such as geographic size and location of area (e.g. proximity to road). We expect to use mixed effects models for both of these analyses. Other research on Reveal implementations has suggested there to be significant power in the tool's ability to deliver data to decision makers and real added impact of Reveal-supported service delivery. Comparative analyses with a tool like Reveal is challenging as its improved measurement capabilities mean there is no true counterfactual using observational data; a small scale RCT is necessary to truly understand the impact. Such research should be considered, with a particular focus on measuring data use and its impact, answering questions such as a) how the use of precision-based, geospatial data tangibly change the way services are planned and delivered, b) how does this influence the quality and coverage of the service and c) how does that translate into impact?

569

RELATIONSHIP BETWEEN MALARIA AND MALNUTRITION IN CHILDREN UNDER FIVE YEARS IN KOILA BAMANAN AN ENDEMIC MALARIA AREA IN MALI

Drissa Konate, Sory I. Diawara, Larissa Denou, Agnès Guindo, Seydou Doumbia, Mahamadou Diakite

MRTC/USTTB, Bamako, Mali

Malaria and malnutrition are common in Mali and constitutes a serious public health problem due their high mortality in children under 5 years. Our preliminaries results show a high prevalence (70% in June 2019) of anemia in children under five years in Koila village were malaria is common. Investigate the relationship between malaria, anemia and malnutrition in children under five years, especially in low-income countries where their prevalence remain high, will be allow to better understand the burden of these coexistence and to propose an appropriate strategy for these disease. The study was conducted in Koila Bamanan village, irrigate area of Markala hydroelectric dam, during a cross sectional survey in November 2019. The data were collected in 233 children involved in this study. The logistic regression model was used to determine the association between malaria and malnutrition. The prevalence of underweight was 14.6%, 40% for stunting & 10.7% for wasting. The prevalence of *Plasmodium* infection was 12.5%, 57.9% of children had at least one episode during malaria transmission season & 52.8% had anemia. After adjusting to confounding variables, malaria episodes number (OR = 2.05, p=0.1) & anemia (OR=6.91, p =0.001) were associated with the underweight. *Plasmodium* infection (OR = 1.74, p=0.2) & anemia (OR=3.28, p=0.001) were associated with the stunting. However, the risk was reduced with polygamy, the age of children, educational level & older mothers. A positive correlation was found between z-score and parasitemia in children with underweight (r=0.2512; p=0.18) or stunting (r=0.2734; p=0.15). However, this correlation was negative in children with wasting (r=-0.0607; p=0.7). Conclusions: the prevalence of malnutrition remains high among children in Koila village, an association between malaria, anemia and malnutrition has been found. More studies are required to better understand the relationship between malaria and malnutrition in order to adopt a common strategy for prevention.

570

CROSS-CULTURAL CLINICAL EXPERIENCE OF SECOND YEAR US MEDICAL STUDENTS IN RURAL COMMUNITIES IN EL SALVADOR, DOMINICAN REPUBLIC AND HONDURAS 2017-2020

Dean Sutphin, Joe Bryan, Etienne Jaime Hinojosa, Harold Garner, Cameron Sumpter, Matt Cannon, Brian Dickens, Brian Dickens, Jeremy White

Via College of Osteopathic Medicine, Blacksburg, VA, United States

The Via College of Osteopathic Medicine (VCOM) with four US campuses in medically underserved regions established permanent global health north-south partnerships continuous primary care clinics in El Salvador, Honduras and Dominican Republic to fulfill the college mission of benevolent outreach and student global health engagement. Our aim is to describe the second-year experience during 30 one-week trips, typically 30 students per trip (900 total) January 2017 to January 2020. Prior to US departure, students receive a medical outreach guide, lectures, medical Spanish workshop, practice procedures, and pack medicines. Clinical and cultural competencies are developed in Spanish-speaking countries by taking patient vital signs and history, focused exams, diagnosis, treatment and prescriptions supervised by US physicians (6 -8 per trip) and 1 - 4 in-country clinic physicians. Students rotate daily in groups of 3-4 with a US physician preceptor and translator assisted by a permanent VCOM in-country licensed physicians to write clinic follow-up or hospital referral. Typically, each day students learn through practice by seeing 5 - 10 of the 100 preselected underserved patients. Services include laboratory (glucose dip stick urinalysis), vision testing and gifting reading glasses, and basic medication. Students use college-developed software, CREDO, to code syndromes using ICD-10. Findings from rural medical outreach include 5292 patients in Tegucigalpa, Honduras; 5389 Veron, Dominican Republic; and 2416 patients in El Salvador. Range of diagnoses across trips and sites include respiratory syndromes 14-20%; Infectious/Parasitic, 12-20%; musculoskeletal, 7-9%; digestive system, 6-10%; other signs/symptoms/lab abnormalities, 7-10%; Nervous system, 6-7%; circulatory, 4-5%; endocrine, 3-4%; ophthalmic 3-4%; skin and subcutaneous, 3%; and genitourinary, 3-5%. In Conclusion, VCOM north-south medical outreach model provides a successful comprehensive Global Health student experience. CREDO is an effective database to record diagnosis, procedures and medicines for documentation and planning future trips.

571

DISTRIBUTION OF CASES AND EVALUATION OF THE SENSITIVITY OF LYME DISEASE EPIDEMIOLOGICAL SURVEILLANCE SYSTEM IN ARMENIA, 2019

Karine Gevorgyan, Armine Ghazazyan, Lusine Paronyan, Lilit Avetisyan, Artavazd Vanyan

NCDCP, Yerevan, Armenia

Two cases of Lyme disease (LD) were reported in Armenia until 2017. After the implementation of the laboratory diagnostic algorithm, 54 cases of preliminary diagnosis of Lyme disease (LD) were registered in 2017-2019. Population seroprevalence study conducted in 2018 detected B. burgdorfery IgG antigens with 31.7% positive results. This study aimed to describe the geographical & seasonal distribution of LD cases, & to evaluate the sensitivity of the surveillance system. EpiInfo was used to analyze statistic data, notification/epidemiological research forms to compare with data of seroprevalence study & vector PCR testing data.

Distribution of cases was mapped using ArcGIS.54 clinical (with laboratory confirmation-45) cases, obtained by surveillance system, were examined. Infection occurred mainly in Syunik-19 (35.1%) & Tavush-12 (22.2%) regions due to the expansion of the geographical range of Ixodes ricinus. The peak of infection occurs in June-July (a significant correlation with the cycle development of ticks to the stage of nymphs & adults, 28% of ticks was infected with B.burgdorfery (PCR test). The peak incidence in July-August (43.4% of cases). Only acute cases with clinical manifestations were included in the routine statistics. Despite the fact that 29(97%) of the seroprevalence study participants reported chronic symptoms of LD & ticks' bites in the past-93 (92%), before this study no one had been tested for LD or included in statistics. LD is underdiagnosed, the surveillance system is not sensitive to chronic diseases cases. Strengthening of diagnostic capabilities, implementations of diagnostic & treatment protocols are necessary in the regions.

572

INITIAL EVALUATION OF THE INBIOS LYME *DETECT*™ IGM-IGG ELISA FOR DETECTION OF LYME DISEASE

Aarthy C. Vallur, Dindo Reyes, Gregory Ireton, James Needham, Syamal Raychaudhuri

InBios International inc, Seattle, WA, United States

Tick-borne diseases are a growing public health problem in the United States (US). The most common disease is Lyme disease, caused by the spirochete *Borrelia burgdorferi*. With an estimated 240000-440000 new infections annually, Lyme disease is an important and expensive emerging illness in the US. Symptoms of early Lyme disease include fever, fatigue, headaches and weakness. Late Lyme disease symptoms such as arthritis, carditis, memory loss and eyesight loss are more prevalent than previously estimated. The Johns Hopkins Bloomberg School of Public Health has estimated that Lyme disease costs the US health care system between $712m- $1.3 b annually. Early detection is important to minimize the debilitating manifestations of late Lyme disease. The diagnosis of Lyme disease currently involves a laborious 2-tier testing algorithm recommended by the CDC. Due to the complex immune response in Lyme disease, diverse immunogenic antigens will be needed in a test to achieve the necessary sensitivity and specificity. InBios developed the Lyme *Detect*™ IgM-IgG ELISA using a recombinant, multi-epitope fusion peptide antigen, VOVO, developed at the National Institutes of Health. In initial evaluation, the ELISA had a sensitivity of 100% and specificity of 95% on Lyme disease and healthy control samples respectively, obtained from commercial sources. When the ELISA was tested on a well characterized panel of samples established by the CDC defined by the stage of Lyme disease symptoms observed, good correlation with the 2- tier algorithm was noted. High sensitivity of 100% was observed for Stage 1 convalescent, Stage 2 neurologic Lyme and Stage 3 arthritic Lyme samples. For the 5 Stage 1 acute samples which fall within the early "window period" of detection before seroconversion, one sample was IgM positive by ELISA while none were positive by the 2- tier algorithm. High specificity was displayed on the healthy controls and other disease samples (92%) in the panel. The InBios Lyme *Detect*™ IgM-IgG ELISA is promising in initial evaluation. We are exploring additional antigens and novel diagnostic algorithms to develop a one-step process for final diagnosis.

573

MISDIAGNOSIS OF CUTANEOUS, ARTICULAR, CARDIAC, NEUROLOGICAL, PSYCHIATRIC AND OTHER MANIFESTATIONS OF TICK-BORNE DISEASES AT THE FIRST VISIT

Don W. Kannangara[1], Roopa Anmolsingh[2], Dhyanesh Pandya[1]

[1]*St Luke's University Health Network, Phillipsburg, NJ, United States,* [2]*St Luke's University Health Network, Betlehem, PA, United States*

In tick endemic areas initial presentation of tick transmitted diseases often mimic common conditions. In a 3-year study of 10 hospitals in our network, articular and cutaneous manifestations were most commonly misdiagnosed. Joint manifestations of Lyme disease were common in young males, majority affecting a single knee and initially misdiagnosed as sports injuries, trauma or sprains and treated with ice, rest, splints, crutches and analgesics. One of 2 misdiagnosed as gout received colchicine. One suspected of septic arthritis had an arthroscopic washout of the knee. Of 5 older patients suspected of osteoarthritis one had arthroscopy, one received prednisone, 2 intra-articular methyl prednisolone or betamethasone and ibuprofen. A patient with erythema migrans misdiagnosed as facial cellulitis was treated with cephalexin followed by vancomycin with no response. Then the diagnosis was revised to contact dermatitis. A chest wall erythema migrans rash in a young female mimicked a poison ivy rash. A 69-year-old male with a patch of erythema in medial left ankle and ankle pain was treated by a family physician with intra-lesional steroids for vasculitis. Later he was western blot positive for Lyme disease. A blistering lesion on the big toe with a suspected staphylococcal infection was culture negative and found to be a Lyme rash. A young female was treated with prednisone by a family doctor for sciatica. The patient continued to be febrile and had an erythema migrans rash on the thigh. The final diagnosis was neuroborreliosis with radiculitis. A middle-aged female with body aches, fever and hearing voices was sent home with acetaminophen for "flu" by the emergency room. She was readmitted with fever, photophobia and auditory hallucinations with an erythema migrans rash on the left shoulder, remembered a tick bite and the diagnosis was revised to Lyme psychosis. A young male who fainted twice at work and initially suspected of a vasovagal attack was later found to have Lyme carditis. Two patients with left flank pain initially misdiagnosed as pyelonephritis had blood smears positive for babesiosis. The left flank discomfort originated from the spleen.

574

EPIDEMIOLOGY OF BABESIOSIS INFECTIONS IN ZHEJIANG PROVINCE, CHINA

Wei Ruan, Xiaoxiao Wang, Jia Zhang, hualiang Chen, linong Yao

Zhejiang Provincial Center for Disease Control and Prevention, Hangzhou, China

Babesiosis is a zoonosis caused by the infection of *Babesia*, which is transmitted by tick bites, blood transfusion, organ transplantation or maternal-neonatal transmission. So far, China has reported about 100 cases, mainly from Heilongjiang province, Yunnan province, Chongqing province, Guangxi province, Shanghai, Xinjiang province and Zhejiang province. Since 2011, five cases of Babesiosis were confirmed in Zhejiang province, China. A retrospective study was conducted to analyze the epidemiology and clinical characteristics of Babesiosis infections. Epidemiology of five Babesiosis infections in Zhejiang province, China between 2011 and 2019 were obtained. Approximately 1mL of finger-prick blood from each patient was collected. Blood smears were used to confirm parasite infection, then PCR was used to double check species. QIAamp DNA Mini kit (Qiagen Inc., Hilden, Germany) was used to extract genomic DNA following instructions. Three of them had a history of recent blood transfusions, and the other two had no clear history of tick bites. Case 1, case3 and case 5 were infected from blood transfusion. Case 2 and case 4 might be infected by tick bites, given farmers from mountain area have more frequent exposure to ticks. *Plasmodium*-like parasites were detected in the red blood cells in all cases (Figure 1), and the sequences of PCR products were consistent with Babesia microti (GenBank: JQ609305). Platelets decreased significantly in five patients, the oral chloroquine for 3 days and intravenous infusion of clindamycin for 10 days showed good effect. Table 1 Epidemiology of Babesiosis infections in Zhejiang province between 2011~2019. In conclusion, Babesiosis cases in Zhejiang province were mainly infected from blood transfusion. followed by tick bites. Further investigation on blood donors and infectious status of *Babesia spp*. in hosts and vectors are need.

575

AFRICAN SWINE FEVER IN THE NORTHERN REGIONS OF CAMEROON: SEROPREVALENCE SURVEY AND SPATIOTEMPORAL ANALYSIS OF OUTBREAKS FROM 2010 TO 2017

Simon Dickmu Jumbo[1], Ranyl Nguena Guefack Noumedem[1], Rodrigue Simonet Poueme Namegni[1], Mohamed Moctar Mouiche Mouliom[2], Julius Awah-Ndukum[3], Abdoulkadiri Souley[1]

[1]*The National Veterinary Laboratory, Garoua, Cameroon,* [2]*School of Veterinary Medicine and Science, University of Ngaoundéré, Ngaoundéré, Cameroon,* [3]*Department of Animal Production Technology, College of Technology, University of Bamenda, Bambili, Cameroon*

African Swine Fever (ASF) is a highly contagious viral disease of domestic pigs and wild boar, with enormous devastating impact on the pig farming in sub-Saharan Africa. It is caused by a large DNA virus of the Asfarviridae family and has as vector soft tick (Ornithodoros moubata). ASF is endemic in Cameroon. This study was carried out to establish the spatiotemporal distribution of ASF between 2010 and 2017 in Adamawa, North and Far North regions of Cameroon. A retrospective study of data relating to these regions was used to analyse outbreaks of ASF from 2010 to 2016 ; 688 blood samples from 185 pig farms were collected and screened for anti ASF antibodies using iELISA tests for the 2017 seroprevalence survey. iELISA positive samples were tested in classical PCR for confirmation. Information on potential risk factors for ASF was obtained by using structured questionnaire. A total of 53 ASF outbreaks were reported and confirmed in the three Northern regions of Cameroon. Over 51.1% of the losses were observed during the first outbreak in 2010 followed by the epizootic outbreak in 2016 (33.4%). The North was the most affected (53.7%) followed by the Far North (43.3%) region, while Adamaoua (3.0%) was the least affected. The 2017 seroprevalence was 5.23% (95% CI [3.57 – 6.89]) at the individual and 10.81% (95% CI [6.34 – 15.28]) at herd level. This seroprevalence was significantly high ($p<0.05$) in pig herds of the Far North 19.23 % (95% CI, [13.55 - 24.91]) compared to those of the North 6.12% (95% CI, [2.67 – 9.57]) and Adamawa 0.58 % (95% CI [0.02 – 1.18]). Region, management of farms system, on-farm slaughter by the owner (OR = 4.60; 95% CI [0.34 - 46.20]; p = 0.014), selling of animals to community or to butchers (OR = 4.82; 95% CI [0.51 - 62.15]; p = 0.010) had significant effect on individual level seropositivity of ASF. The viral antigen was not detected by PCR. This study showed that ASF cases have decreased significantly in the northern regions of Cameroon following the epizootic 2010 outbreaks. The findings predict a better future for the pig farming in the regions through the enforcement of strategic control measures.

576

BARTONELLA SPECIES IN CAMBODIA, GHANA, LAOS, AND PERU: RESULTS FROM SERO- AND VECTOR- SURVEYS

Kristin E. Mullins[1], Enrique Canal[2], Pidor Ouch[3], Didot Prasetyo[4], Janice Tagoe[5], Matthew Robinson[6], Mark Simons[7], Paul Newton[8], Allen Richards[9], Christina Farris[7]

[1]*University of Maryland School of Medicine, Baltimore, MD, United States,* [2]*NAMRU-6, Lima, Peru,* [3]*NAMRU-2, phnom penh, Cambodia,* [4]*NAMRU-2, Phnom Penh, Cambodia,* [5]*NARMU-3 det Ghana, Baltimore, MD, United States,* [6]*LOMWRU, Vientiane, Lao People's Democratic Republic,* [7]*Naval Medical Research Center, Silver Spring, MD, United States,* [8]*Oxford University, Oxford, United Kingdom,* [9]*Uniformed Services University, Bethesda, MD, United States*

Bartonella species are fastidious gram-negative vector-borne bacteria with a wide range of mammalian reservoirs. The extent of human exposure to Bartonella species, whether it be *Bartonella quintana, B. henselae, B. bacilliformis*, or those less commonly associated with human disease, has yet to be fully understood. To this end, residual sera from participants enrolled in undifferentiated fever studies in Cambodia, Ghana, Laos, and Peru were screened for the presence of IgG antibodies against *B. quintana* and *B. henselae*, using the FOCUS diagnostics Dual Spot- *Bartonella* IgG Immunoflourscence assay (titer of 1:64 as cut-off for positives). Forty-eight patients with suspected *B. bacilliformis* exposure or infection, due to a 2003 outbreak of *B. bacilliformis* in Peru, were screened to assess cross-reactivity of assay with other *Bartonella species*. Overall 75% (36/48) of the samples were positive, including 10/13 samples from patients with confirmed *B. bacilliformis*. Additionally, 38% (79/206), 22% (44/200), 56% (101/180), and 57% (57/100) of samples from Peru, Laos, Cambodia, and Ghana were positive. Further, ectoparasites pools from the Cambodia, Laos, and Peru were tested using real-time PCR methods for the presence of *Bartonella* species. Of the sandfly pools tested, 0/192 were positive from Peru; 15/140 flea pools were positive from Cambodia; while 0/106 ticks, 0/22 fleas, and 1/2 louse pools from Laos tested positive for *Bartonella* species. The serologic results indicate that human exposure to *Bartonella* species is occurring in these countries. Evidence of *Bartonella* in fleas from Cambodia supports the possibility that humans are exposed to *Bartonella* through this traditional vector, however *Bartonella* species were not found in fleas or ticks from Laos, or sandflies from Peru. This could account for the lower positive serology among the population in Laos and the localized nature of *Bartonella bacillformis* infections in Peru. Whether *Bartonella* species was the cause of any cases of undifferentiated fever in these subjects is unclear, however, *Bartonella* as a human pathogen warrants further investigation.

577

INTERESTS IN BIOLOGICAL CONFIRMATION OF MALARIA DIAGNOSIS IN DECISION MAKING FOR MALARIA TREATMENT IN THE DEMOCRATIC REPUBLIC OF THE CONGO

Laishe Brigitte

Ministère de la santé publique, Kinshasa, Democratic Republic of the Congo

LAISHE B[1], MUSEMA GM[2], MUKOMENA S[1,3], LIKWELA J[4] 1.National Malaria Control Program, DR Congo, Ministry of Health;2.Kinshasa School of Public Health, University of Kinshasa;3.School of Public Health of Lubumbashi;4.Faculty of Medicine of University of Kisangani;
INTRODUCTION:

Malaria treatment was based on the syndromic approach, before 2010, we consider fever equal to malaria and treated as such. And yet, it is known that fever is common symptom of several diseases, especially in children under 5 years old. In 2010, WHO recommended to endemic countries malaria biological confirmation prior to treatment of suspected malaria cases. The DRC's national malaria control program had taken ownership of this initiative and had made it one of its strategies to fight. This study aims to demonstrate the interest of biological confirmation of malaria diagnosis in management of this condition, in order to prevent occurrence of *Plasmodium* resistant strains to treatment. We carried out a retrospective study based on data from 2016 and 2017 annual reports of National Malaria Control Program of 26 Health Districts, on all suspected cases of malaria subjected to Rapid diagnostic test (RDT), and thick drop. In 2016, there were approximately 21,569,754 suspected malaria cases were enregistred, of which 86.3% were undergoing RDT and 72% were positive. Similarly, 13% thick droplets made whose 8.5% were positive. In 2017, out of a total of 21 959 428 malaria suspected cases, 86.4% cases were submitted to RDT and 9.4% thick droplets made. After reading, 73.3% and 6% cases were positive, respectively for RDT and microscopy. In conclusion, these data demonstrate prove the impact of biological confirmation of the diagnosis of malaria. The early management of malaria through biological confirmation prevents the occurrence of epidemics caused by strains resistant to plasmodium to antimalarial treatments.

578

FILLING IN THE GAP: FIRST STATE-WIDE TICK (ACARI: IXODIDAE) SURVEY IN SOUTH CAROLINA

Kyndall C. Dye-Braumuller, Melissa S. Nolan

University of South Carolina, Columbia, SC, United States

During the past decade, tick-borne disease incidence has increased, and the southeastern US is emerging as a hotspot of concern. According to the South Carolina Department of Health and Environmental Control (SC DHEC), approximately 74% of all reported vector-borne disease cases from the past 5 years have been tick-borne. With no formal tick surveillance, the threat of tick-borne disease is only addressed symptomatically and retroactively after disease onset. In addition, there is a paucity of research in the state regarding ticks and the pathogens they carry; the most recent comprehensive tick surveillance studies in South Carolina were conducted in the 1970s. Research and government groups in neighboring states, Tennessee, Georgia, and North Carolina, conduct tick surveillance, yielding crucial information regarding risk areas for residents, pathogen occurrence, and movement of invasive species, such as the Asian Longhorned tick, *Haemaphysalis longicornis*. Our group began the first state-wide tick surveillance program in March 2020 to identify, monitor, and research tick species and tick-borne pathogens. Tick-borne disease data from SC DHEC was utilized to select public parks for tick collecting within the four major regions of the state: Upstate, Midlands, Pee Dee, and Low Country. Tick dragging and CO_2-baited traps were placed in state and local parks and privately-owned land bimonthly, at minimum. All ticks were speciated and processed for pathogen testing. *Ixodes* and *Amblyomma* species were the most frequently collected, all life stages except larvae and eggs were collected, and pathogens of public health importance were detected among our four regions. *Ixodes scapularis*, the blacklegged tick, was determined as "established" in multiple counties where it was only "reported" previously or no data was available. The results of this survey have informed state public health officials to prepare for emerging tick-borne disease threats and invasive species.

581

A SALIVARY PROTEIN OF *AEDES AEGYPTI* PROMOTES DENGUE-2 VIRUS REPLICATION AND TRANSMISSION

Shin-Hong Shiao

National Taiwan University, Taipei, Taiwan

Dengue is the most prevalent arthropod-borne viral disease in humans, yet still no effective medication or vaccine is available. Previous studies indicated that mosquito salivary proteins may influence the infection of dengue virus in the mammalian host. However, the function of specific salivary proteins on DENV replication in *Aedes aegypti* mosquito remains largely unknown. In this study, we investigated the effect of a salivary protein, named AaSG34, on DENV serotype 2 (DENV2) replication and transmission. We reveal that transcripts of AaSG34 were upregulated in the mosquito salivary glands after an infectious blood meal with DENV2. We further disclosed that transcripts of the dengue viral genome and envelop protein in the mosquito salivary glands were significantly diminished after infectious blood meal in the absence of AaSG34. To clarify the effect of AaSG34 on DENV2 transmission, *Stat1*-deficient mice were used. We reveal that while intradermal inoculation of infectious mosquito saliva induced hemorrhage development in *Stat1*-deficient mice, saliva from the AaSG34-silenced mosquitoes lost the ability to induce hemorrhage, suggesting that the AaSG34 enhances DENV2 transmission. Taken together, this is the first report to reveal that the AaSG34 promotes DENV2 replication in the mosquito salivary glands as well as enhancing transmission of the virus to mammalian host.

582

GENETIC SILENCING OF PYRUVATE KINASE IN *AEDES AEGYPTI* FEMALES IMPACTS SURVIVAL, GLUCOSE OXIDATION, AND AMMONIA DETOXIFICATION

Natthida Y. Petchampai[1], Jun Isoe[2], Thomas D. Horvath[3], Shai Dagan[4], Lin Tan[3], Philip L. Lorenzi[3], David H. Hawke[3], Patricia Y. Scaraffia[1]

[1]*Tulane Unversity, New Orleans, LA, United States,* [2]*The University of Arizona, Tucson, AZ, United States,* [3]*The University of Texas MD Anderson Cancer Center, Houston, TX, United States,* [4]*Israel Institute for Biological Research, Ness Ziona, Israel*

The final and rate-limiting step of glycolysis is catalyzed by pyruvate kinase (PK, EC 2.7.1.40), an enzyme that catalyzes the transfer of a phosphate group from phosphenolpyruvate to ADP, yielding pyruvate and ATP. A recent *in vitro* characterization of a recombinant pyruvate kinase (PK) from *Aedes aegypti* mosquitoes demonstrated that the enzyme is uniquely regulated by multiple allosteric effectors. To elucidate the functional role of PK in *A. aegypti in vivo*, RNA interference-mediated silencing of PK was performed. Interestingly, knockdown of PK enhanced survival rate of mosquitoes fed with sugar, water, or blood/water. However, RNAi-mediated PK reduction in blood-fed females that were continuously supplied with sucrose did not affect mosquito survival. Further analysis revealed up-regulation of genes encoding NADP-malic enzyme-1 and phosphoenolpyruvate carboxykinase-1 in the fat body, phosphoglycerate dehydrogenase in the thorax, and glutamate dehydrogenase in both tissues of PK-deficient mosquitoes. To evaluate whether PK deficiency impacts the abundance or kinetics of metabolites involved in specific glucose pathways, Krebs cycle, and pathways associated with ammonia fixation, assimilation and detoxification, we fed 4-day-old dsRNA-injected females a blood meal supplemented with [1,2-$^{13}C_2$]-glucose, and examined the incorporation of the ^{13}C-atoms from [1,2-$^{13}C_2$]-glucose into several metabolites in mosquito whole body and excreta using advanced mass spectrometry methods. Our data reveal a significant reduction of metabolite abundance in glycolysis, pentose phosphate pathway, Krebs cycle, and ammonia detoxification pathways. Taken together, our results provide evidence that PK plays a key regulatory role in the metabolic homeostasis of *A. aegypti* females.

585

PAVING THE WAY FOR THE MOSQUITO STERILE MALE TECHNIQUE: CHARACTERIZING SEX BIASED EXPRESSION OF PUTATIVE SPERMATOGENESIS AND SEX DETERMINATION GENES IN *ANOPHELES ALBIMANUS*

Claudia Paiz[1], Mabel Taracena[2], Elena Ortiz[1], Andrea Ramos[1], Quetzali Gonzalez[1], Ana L. Melendez[1], Stefany Argueta[1], Gabriela Lara[1], Claudia Alfaro[1], Paula Villatoro[1], Norma Padilla[1], Ellen Dotson[3], **Pamela M. Pennington**[1]

[1]*Universidad del Valle de Guatemala, Guatemala, Guatemala,* [2]*2Centers for Disease Control and Prevention, Atlanta, GA, United States,* [3]*Centers for Disease Control and Prevention, Atlanta, GA, United States*

Insecticide resistance poses a threat to the malaria elimination goal in Central America. Mosquito suppression through the Sterile Insect Technique (SIT) could provide a sustainable control tool. In the 1970's, *Anopheles albimanus* wild populations were controlled in El Salvador using chemosterilization. Current mosquito SIT technologies use irradiation for sterilization and separate females through pupal size sorting. However, both methods are suboptimal in anophelines. Silencing spermatogenesis and sex determination genes by oral mediated RNA interference (RNAi) has been proposed to complement SIT programs. The first step towards RNAi based sterilization and female elimination is to evaluate potential gene silencing targets. We first looked for genes with low variability in expression as qPCR stable reference genes. Three genes were tested: ribosomal protein S4 (*RpS4*), ribosomal protein L49 (*RpL49*) and the enzyme glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*). The gene GAPDH was discarded due to its variability but *RpS4* and *RpL49* showed

stable expression. We characterized by qPCR the relative expression of orthologs of spermatogenesis-related genes (the gene for the boule RNA binding protein [*bol*] and the zero population growth [*zpg*] gap-junction protein, important in gametogenesis) and sex determination genes (the doublesex gene [*dsx*] and transformer 2 [*tra*-2] involved in upstream splicing*),* in various stages from third instar (L3) to male and female adults of *A. albimanus*. Expression of *zpg* was female-biased in adults but not pupae. We did not observe sex specific expression of either *bol* or *tra-2* in any stage. Expression of *dsx* significantly increased in male adults compared to L3. In addition, we identified two *dsx* female biased isoforms (*dsxF* long and short). We observed greater expression of *dsxF* long in females, compared to *dsxF* short. Taking these results together, we suggest that *zpg* and *dsx* show the highest potential as silencing targets due to their significant sex biased expression.

586

PHENOTYPIC, GENOTYPIC AND METABOLIC CHANGES THAT OCCUR DURING THE SELECTION PROCESS OF PYRETHROID-RESISTANCE IN *ANOPHELES GAMBIAE*

Maxwell G. Machani[1], Eric Ochomo[1], Guofa Zhou[2], Andrew Githeko[1], Guiyun Yan[2], Yaw Afrane[3]

[1]*Kenya Medical Research Institute, Kisumu, Kenya,* [2]*Program in Public Health, College of Health Sciences, University of California, Irvine, CA, United States,* [3]*Department of Medical Microbiology, College of Health Sciences, University of Ghana, Research Institute, Accra, Ghana*

Knowledge on the insecticide resistance mechanisms and their evolution is necessary for the design and implementation of insecticide resistance management strategies. In the process of establishing a deltamethrin-resistant *Anopheles gambiae* colony, we documented the phenotypic, genotypic and metabolic changes that occur in pyrethroid-resistant mosquitoes when selection pressure is maintained versus when there is no selection pressure. F_1 *Anopheles gambiae* s.s females raised from adults collected from the field were selected for deltamethrin resistance using WHO standard protocol for 13 generations. Resistant markers and metabolic enzymes were characterized. Phenotypic resistance increased steadily in the selected strain (F_1 to F_{13}) (Mortality range: 42-29%. The unselected-strain progressively became more susceptible to deltamethrin over time (F_1-$F_{18)}$ (Mortality range; 42-97%). The frequency of Vgsc-1014S and 1014F in the F_1 was 0.94 and 0.16 respectively. The frequency of 1014S became fixed (1) in the selected population at F_4 and 1014F alleles increased to 0.43. For the unselected strain, the frequency of 1014S was 0.96 and 0.97 at F_{10} and F_{14} respectively. The frequency of 1014F declined to 0.01 at F_{10} compared to the parent population and was not detected at F_{14}. There was a significant increase in monooxygenases enzyme activity by 2-fold in the selected strain. There was a significant reduction in monooxygenases activity by 2-fold in unselected strain. The results show that physiological resistance increased with continued selection pressure and declined in the absence of selection pressure. An increase in monooxygenases enzyme activity and 1014F in the selected strain suggest continuous insecticide pressure will select for markers of insecticide resistance. Regular monitoring of insecticide resistance in disease vectors that could inform changes in disease control strategies is highly recommended.

587

EFFECTS OF SHORT-TERM WEATHER ON THE HOST-SEEKING ACTIVITY OF WEST NILE VIRUS VECTORS AND IMPLICATIONS FOR INTEGRATED VECTOR MANAGEMENT

Pascale Stiles[1], Gurmanpreet Kaur[1], Mary Sorensen[2], Mario Boisvert[2], Jake Hartle[2], Marcia Reed[3], Sarah Wheeler[3], Christopher Barker[1]

[1]*University of California, Davis, Davis, CA, United States,* [2]*Placer Mosquito and Vector Control District, Roseville, CA, United States,* [3]*Sacramento-Yolo Mosquito and Vector Control District, Elk Grove, CA, United States*

Vector control programs that aim to reduce West Nile virus (WNV) disease are guided by entomological risk metrics derived from counting and testing adult female mosquitoes from CO_2-baited traps. Weather, including temperature, wind speed, and relative humidity, is known to affect the population dynamics of these species, but little attention has been paid to the potential for nightly weather conditions to induce bias in estimates of mosquito host-seeking activity. Instead, trap counts are simply averaged to estimate vector abundance in an area. We collaborated with local vector control districts to collect mosquitoes from 10 automated counter traps across three counties within the rice-growing region of the Sacramento Valley during the summer of 2019. From each collection, we counted adult *Culex tarsalis* females, the predominant WNV vector in the study area, then related these counts to the weather conditions experienced at each site during the same time periods. Data were aggregated for each overnight observation period, which is the most commonly used unit for estimating adult mosquito abundance. Overall, *Cx. tarsalis* activity was greatest during periods of warmer average temperatures during host-seeking periods, lower average wind speed, and cooler daily maximum temperatures. These relationships were consistent across the different study sites, which suggests that a generalizable correction for these ephemeral effects on mosquito activity could be applied to more accurately estimate mosquito abundance. Ongoing studies will evaluate the effects of weather on timing of activity during evening crepuscular periods using trap counts within 15-min intervals. Taken together, the findings from this study will help explain and adjust for an important source of variation in a routinely monitored component of arbovirus risk assessment programs. The results will aid vector control programs in both interpreting WNV transmission risk based on mosquito abundance estimates and timing the application of broad-scale adult mosquito control measures for maximum impact on vector populations.

588

NEW METHODS FOR MODELING *ANOPHELES GAMBIAE* S.L. MOVEMENT WITH ENVIRONMENTAL AND GENETIC DATA

Tomás M. León[1], Héctor M. Sánchez Castellanos[1], Yoosook Lee[2], John M. Marshall[1]

[1]*University of California, Berkeley, Berkeley, CA, United States,* [2]*University of California, Davis, Davis, CA, United States*

Models that simulate the effects of interventions on malaria vectors and transmission make assumptions about how mosquitoes move in the environment, such as isotropic behavior and no sex-related differences. These are applied to dispersal between households and villages and processes such as host-seeking and oviposition. Most models use mathematically convenient dispersal kernels based on these assumptions given the paucity of available data to better parameterize mosquito movement and the increase in complexity required. Consequently, there are few available methods to explore the effects of landscape and environmental factors on dispersal patterns. Understanding these patterns is key to optimizing control strategies, particularly for genetic control methods that involve releasing modified mosquitoes that compete with the natural mosquito population. We present a framework for modeling *Anopheles gambiae* s.l. movement mechanistically using available mark-release-recapture, biological, and ecological data and describe how it can be tailored for different locations and scenarios. We demonstrate its use for São Tomé and Príncipe and the Comoros, two candidate field

sites for genetic control trials. Furthermore, we show the effects on these islands of elevation, land use, village/city proximity, and wind on predicted dispersal kernels and the implications for mosquito population dynamics. The resultant dispersal kernel is unique to the landscape of interest and is easily calibrated to field data measurements. Finally, we compare these results with genetic methods for inferring dispersal and connectivity between different mosquito populations on the islands and suggest future directions for the synthesis of these two data streams.

589

ENTOMOLOGICAL INVESTIGATIONS OF MALARIA OUTBREAKS IN SOUTHEASTERN MADAGASCAR HIGHLIGHT THE ROLE OF THE SECONDARY MALARIA VECTOR *ANOPHELES COUSTANI* AND SUBSTANTIAL OUTDOOR BITING

Thiery Nepomichene[1], Tsarasoa Andrianinarivomanana[1], Rico Randrenjarison[1], Tsiry Rakotomanga[2], Jocelyn Ratovonjato[2], Romain Girod[1]

[1]*Institut Pasteur of Madagascar, Antananarivo, Madagascar,* [2]*National Malaria Control Program, Ministry of Public Health, Antananarivo, Madagascar*

In February 2019, a malaria outbreak occurred in Farafangana where long-lasting insecticidal nets (LLIN) had been distributed and indoor residual spraying (IRS) conducted a few months earlier. Ivohibe, where LLINs had also been distributed, had an outbreak in January 2020. Entomological investigations were conducted in these two districts. Two mosquito collectors per night conducted indoor and outdoor human landing catches in up to four homes for three consecutive nights in each of five affected villages. Human biting rates (HBR) were calculated and mosquitoes identified morphologically and using PCR. ELISA was used to detect *Plasmodium* sporozoite antigens and PCR to confirm positives. In Farafangana, *An. coustani, An. funestus, An. gambiae* and *An. squamosus* represented 92.1% (N=1,371/1,488) of anopheline mosquitoes in the two villages investigated. The HBR ranged from 0.7 bites per human per night (bhn) for *An. squamosus* indoor to 11.6 bhn for *An. coustani* outdoor. All species except *An. funestus* exhibited predominantly exophagic behavior with >77% of bites occurring outdoors; 1.7% were *P. falciparum*-positive, nearly two-thirds of which were *An. coustani* collected outdoors. In the three Ivohibe villages, *An. coustani, An. arabiensis, An. squamosus, An. flavicosta* and *An. gambiae* represented 91.8% (N=445/485) of anopheline mosquitoes. The HBR varied from 0.4 bhn for *An. gambiae* indoor to 2.0 for *An. coustani* indoor. An exophagic rate <59% was observed in all species, including for *An. coustani* (47.1%). Ten (2.1%) mosquitoes harbored sporozoite antigens and *Plasmodium* DNA, all of which were *An. coustani* positive for *P. vivax*. Indoor and outdoor biting and parasite transmission is occurring in outbreak settings in southeastern Madagascar; *An. coustani* is an important local malaria vector. In Farafangana, vectors were predominantly exophagic, possibly a consequence of LLIN distribution combined with IRS. In Ivohibe, where only LLINs had been distributed, 17 months prior, there was only a trend toward exophagy. Vector strategies targeting both indoor and outdoor anopheline biters are needed.

590

WHY ARE SOME MOSQUITO SPECIES INVASIVE?

Daniel A. Peach, Benjamin Matthews

University of British Columbia, Vancouver, BC, Canada

Mosquitoes are the world's deadliest animals due to the pathogens that they spread. Some species of mosquito have successfully expanded beyond their native range to invade new habitat in many regions of the world. The goal of our research is to understand what makes some mosquitoes so adept at invading new areas.There are many traits that might affect the ability of a mosquito to establish itself within new territory, including oviposition behavior, host-feeding, physiological tolerances, previous distribution patterns, and ecological interactions. Using the yellow fever mosquito, *Aedes aegypti*, and the coastal rock pool mosquito, *Ae. togoi*, our research investigates these traits and their phenotypic variability by exploring the genetics, behavior, ecology, and biogeography of invasive mosquitoes.We use a multi-layered approach to explore within- and among- species variation that allows some organisms to become widespread and invasive, while closely related species remain narrowly restricted specialists. At the habitat scale, we use machine learning techniques to create species distribution models of *Ae. togoi* habitat to identify environmental determinants of its distribution. We use molecular blood-meal identification to investigate *Ae. togoi* host-feeding patterns. In the lab, we ask how *Ae. togoi* tolerates environmental conditions, including salinity of breeding water and temperature tolerances and perform experiments to assess *Ae. togoi* breeding site selection and oviposition behavior.To understand the genomic basis of these traits, we propose to propagate strains of each species by selecting for specific oviposition behaviors and to use re-sequencing and transcriptional profiling approaches to identify specific genetic features that correlate with behaviors. Ultimately, we plan to test candidate genomic loci using CRISPR/Cas9 to generate loss-of-function mutations and to swap alleles to gain a mechanistic understanding of this variability. Together our work hopes to elucidate the factors driving the spread of invasive mosquitoes (e.g. *Aedes aegypti*) in order to aid efforts to combat mosquito-borne disease.

591

AEDES AEGYPTI BREEDING IN TRASH: BARRIERS TO EFFECTIVE TRASH COLLECTION, DISPOSAL AND RECYCLING IN UKUNDA, KWALE COUNTY KENYA

Francis M. Mutuku[1], Lydia Kibe[2], Amy Krystosik[3], Mumini Dzoga[1], Scholastica Ratanya[1], Gathenji Njoroge[4], Jenna Forsyth[3], A. Desiree LaBeaud[3]

[1]*Technical University of Mombasa, Mombasa, Kenya,* [2]*Kenya Medical Research Institute, Kilifi, Kenya,* [3]*Stanford University, San Fransciso, CA, United States,* [4]*University of California Berkeley, Berkeley, CA, United States*

Dengue virus (DENV), chikungunya virus (CHIKV), and Zika virus (ZIKV) are three important arboviruses with wide geographic spread and increasing impact on vulnerable human populations that are spread by the same mosquito vector (*Aedes aegypti). Ae. aegypti* preferentially breed in containers, often trash or unused containers, within human settlements. Studies have demonstrated that poor waste management leads to accumulated solid waste, especially plastics, and is associated with both transmission and risk of dengue and chikungunya. Intervention studies in Asia have demonstrated the potential to control *Ae. aegypti* by regular plastic wastes collection and disposal. In the present study, we assessed the feasibility of community-based trash recycling as an *Ae. aegypti* control intervention in Ukunda, Kwale County, Kenya. We explored barriers to trash collection, disposal and recycling. We conducted 3 semi-structured in-depth interviews with policy makers and local entrepreneurs and 13 focus group discussion with different groups of community members. Discussions focused on types of trash, perceptions toward trash, stakeholders in trash collection, challenges of managing trash in the community and associated vector-borne disease risks. Low public environmental awareness, insufficient waste management infrastructure, weak enforcement and ineffective policy implementation were identified as key barriers to efficient collection, disposal and recycling of wastes in Ukunda. The community perceived garbage as an important problem but observed that there is little knowledge regarding the ways individuals could contribute to solving it. The low environmental awareness was also associated with lack of desire to engage in trash management resulting in low community participation. These results suggest an urgent need for local authority to collaborate with the community to improve access and availability of both primary and secondary storage facilities. Importantly, continuous environmental education is recommended as a key strategy for effective trash collection, disposal and recycling.

593

VECTOR CONTROL INTERVENTIONS DISPROPORTIONATELY AFFECT *ANOPHELES GAMBIAE S.L* AND *AN. FUNESTUS S.L* MOSQUITO DENSITIES FROM THREE SITES IN UGANDA

Henry Ddumba Mawejje[1], Maxwell Kilama[1], Sarah Staedke[2], Moses Kamya[1], Martin J. Donnelly[3], Christian Bottomley[2], Grant Dorsey[4], Jo Lines[2]

[1]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [2]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [3]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [4]*University of California, San Francisco, California, San Francisco, CA, United States*

In the past decade, long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) have been scaled up. Here we examine the disproportionate impact of LLINs and IRS on *Anopheles* mosquito density. Mosquito collections were done routinely in 3 sites from October 2011 to May 2016 in 100 randomly selected households per site using CDC light traps. The study sites include: Walukuba, Kihiihi and Nagongera. In Walukuba and Nagongera, universal LLIN distribution was implemented in November 2013 and in Kihiihi-June 2014. Within the study period, only Nagongera received IRS with 3 rounds of bendiocarb. Results were stratified by intervention period. In Nagongera: LLINs were associated with a 2.5-fold reduction in *An. gambiae s.s* (28.0 to 11.2, p=0.003) and *An. Arabiensis* (9.18 to 3.65, p=0.004) with a 1.5-fold reduction in *An. funestus s.l* (3.90 to 2.56, p=0.07). Following the first round of IRS, a 10-fold reduction in *An. gambiae s.s* (28.0 to 2.71, p<0.001); a 1.7-fold reduction in *An. arabiensis* (9.8 to 5.44, p=0.07) and a 39-fold reduction in *An. funestus s.l* (3.9 to 0.10, p<0.001) was observed. The subsequent 2nd and 3rd rounds of IRS showed a 164-fold reduction in *An.gambiae s.s* (28 to 0.17, p<0.001); a 4.6 fold reduction in *An.arabiensis* (9.18 to 2, p<0.001); and a 650-fold reduction in *An.funestus s.l* (3.90 to 0.006, p<0.001). In Walukuba: There was no difference in *An. gambiae s. s* (0.34 to 0.33, p=0.21) and *An. arabiensis* (0.58 to 0.35, p=0.45) mosquito density post LLIN distribution. However, there was a 3.5-fold reduction in *An. funestus s.l* (0.07 to 0.02, p=0.001). In Kihiihi: There was a 1.6-fold reduction in *An. gambiae s. s* (4.0 to 2.46, p=0.02), insufficient numbers of *An. arabiensis* and *An. funestus s.l* were collected. The differential impact of LLINs and IRS on Anopheles mosquito density was observed, with the steepest decline observed in *An. funestus s.l* and *An. gambiae s.s*. Whilst the vector control interventions caused decline in mosquito density in all the Anopheles mosquito species, the magnitude of this decline was not uniform.

594

ECOLOGY OF *ANOPHELES* MOSQUITO LARVAE IN DIFFERENT ECOLOGICAL ZONES IN GHANA

Isaac A. Hinne[1], Simon K. Attah[1], Akua O. Forson[2], Yaw A. Afrane[1]

[1]*Department of Medical Microbiology, School of Medicine and Dentistry, College of Health Sciences, University of Ghana, Accra, Ghana,* [2]*Department of Medical Laboratory Sciences, School of Biomedical Sciences, College of Health Sciences, University of Ghana, Accra, Ghana*

This study characterized the breeding habitats of *Anopheles* mosquitoes in rural communities in different ecological zones in Ghana during the dry and wet seasons. We studied the spatio-temporal distribution, species composition and abundance of larval Anopheles mosquitoes in Anyakpor (Coastal Savanna area), Duase (Forest area), Libga, Pagaza and Kpalsogu (Sahel Savanna area). Larvae were collected using standard dippers and were raised in the insectary for identification. A total of 383 breeding habitats were recorded during the study, 140 in the dry season and 243 in the rainy season. There were 29.29 % (41/140) and 29.63 % (72/243) of habitats that contained *Anopheles* immatures during the dry and rainy seasons respectively. Out of a total of 6,305 mosquito immatures collected, 2,152 (34.13 %) were Anophelines with 628 (29.18 %) recorded in the dry season whereas 1,524 (70.82 %) were collected in the rainy season. The Anophelines were made up of 2,128 (98.88 %) *An. gambiae s.l.*,16 (0.74 %) *An. rufipees* and 8 (0.37 %) *An. pharoensis*. Dug-out wells had the highest larval densities (3.18 larvae/dip) followed by tyre tracks (2.99 larvae/dip), hoofprints (1.96 larvae/dip), concrete wells (1.85 larvae/dip), footprints (1.69 larvae/dip), swamps (1.36 larvae/dip), furrows (1.29 larvae/dip), man-made ponds (1.01 larvae/dip) and puddles (1.01 larvae/dip), drainage ditches (0.78 larvae/dip) and natural ponds (0.45 larvae/dip). Larval habitat types influenced the presence of larvae as well as larval densities ($p < 0.001$). While the land-use type affected the presence of *Anopheles* larvae ($p = 0.001$), it did not influence their larval densities. Vegetation cover, on the other hand, did not determine the presence of *Anopheles* larvae but influenced larval densities ($p < 0.05$). The data generated from this study is useful in informing vector control through larval source management.

596

DEVELOPING A LANDSCAPE TYPOLOGY SCHEME TO ASSESS ENVIRONMENTAL AND SOCIAL RISK FACTORS FOR DENGUE IN TROPICAL URBAN SETTINGS

Pallavi Amritha Kache[1], Isabella Rodas Arango[2], Andrea Sarmiento Ospina[3], Heriberto Vásquez Serna[3], Mauricio Santos Vega[2], Maria A. Diuk-Wasser[1]

[1]*Columbia University, New York, NY, United States,* [2]*Universidad de los Andes, Bogotá, Colombia,* [3]*Secretary of Health of Ibagué, Ibagué, Colombia*

Worldwide, the burden of dengue fever has increased over the past 30 years, owing, in part, to the geographic expansion of *Aedes* spp. mosquitoes. In the absence of a widely available vaccine or treatment options, dengue incidence is mitigated through control of *Aedes* spp. and vector habitat management. We hypothesize that vector control can be optimized by accounting for the within-city ecological and social processes that generate heterogeneities in transmission. As a proof of concept, we present a statistical typology analysis for Ibagué, Colombia, a city that has an average annual incidence rate of 164 cases per 100,000 population (2007-2018) and consistently reports among the highest case counts in the country. This analysis can be used to capture the spatial heterogeneity in the biophysical and socio-demographic features of the urban landscape. To conduct the typology analysis, we: 1) Collated environmental, demographic, and socio-economic attributes from satellite imagery and publicly available census datasets and extracted values for each 100-meter grid cell in Ibagué's urban extent. 2) Used a principal component analysis to determine the dimensions of variability and relative contribution of each attribute to this variability. We found that the first and second dimensions explained 63.2% of the variation across our initial set of predictor variables, and included attributes such as housing density, socio-economic strata, and the availability of running water. 3) Iteratively ran a hierarchical cluster analysis and determined the optimal number of clusters; and 4) Assigned each cell into k=7 clusters, where each cluster corresponds to a unique urban typology. Using a negative binomial regression model, we then examined the relative importance of urban typology, temperature, and precipitation in determining *Aedes aegypti* larval indices and dengue incidence at a monthly time-step for 2013-2019. The resulting typology designations, further developed in collaboration with the Secretary of Health of Ibagué, can help define operational subdivisions for mosquito surveillance, testing, and control at a sub-neighborhood scale.

597

ENHANCED DIGITIZATION TECHNIQUES UNLOCK SECRETS FROM THE NATIONAL MOSQUITO COLLECTION

Sarah E. Yount

University of Georgia, Athens, GA, United States

This study establishes a novel approach to digitize glass slide-mounted mosquito specimens housed within the United States National Museum (USNM) Culicidae collection. Many glass slide-mounted specimens within the USNM collection have valuable associated data related to the

immature habitats of mosquito species of medical importance. This vast resource has thus far had limited impact to inform geospatial analyses and more detailed habitat descriptions because the data is not in a digital format. The protocol developed during this project ensures that each specimen is cataloged, imaged and georeferenced, greatly enhancing the utility of the data associated with each specimen. Here we present the findings of a digitization pilot project focusing on three mosquito species of medical importance to the United States: *Aedes albopictus, Culex erraticus*, and *Anopheles quadrimaculatus*. In addition to the results of the digitization efforts, we also detail a novel methodology for rapid digitization of slide-mounted specimens.

598

EFFECTS OF IRRIGATION SYSTEM ON *ANOPHELES ARABIENSIS* VECTORIAL CAPACITY IN WESTERN KENYA

Benyl Ondeto[1], Daibin Zhong[2], Harrysone Atieli[3], Guofa Zhou[2], Mingchieh Lee[2], Simon Muriu[4], David Odongo[1], Horace Ochanda[1], Andrew Githeko[5], Guiyun Yan[2]

[1]*University of Nairobi, Nairobi, Kenya,* [2]*University of California at Irvine, Irvine, CA, United States,* [3]*Maseno University, Kisumu, Kenya,* [4]*Pwani University, Mombasa, Kenya,* [5]*Kenya Medical Research Institute, Kisumu, Kenya*

Irrigated agriculture is usually undertaken to curb food insecurity and alleviate poverty. However, it affects the land use patterns and modifies the environment thereby impacting on density of disease vectors populations. The aim of the current study was to assess the effect of irrigation on the *Anopheles arabiensis* malaria transmission potential. The study was conducted in irrigated and non-irrigated sites of Homa bay county, western Kenya from January 2019 to June 2019. Seasonal variations in abundance and behavioural patterns of *An. arabiensis* were evaluated by collections using five sampling methods. Resting and feeding behaviour was evaluated using pyrethrum spray catches (PSCs) (indoor), clay pots (outdoor) and pit shelters (outdoor). Host-seeking behavior and biting rates were assessed using human landing catches (HLCs) and CDC light-traps. A total of 2,815 *An. arabiensis* were collected with irrigated zones reporting higher densities (3.6± 0.2) than non-irrigated areas (0.2± 0.02). Although there was significant seasonal influence on *An. arabiensis* density in non-irrigated sites (F_{710}=25.1, p<0.05), their variation in irrigated sites was not significant (F_{789}= 2.7, p>0.05). Indoor and outdoor resting of *An. arabiensis* was not influenced by irrigation. The propensity to feed on humans was higher in irrigated sites (Human Blood Index {HBI} =0.03) than in non-irrigated sites (HBI=0) while sporozoite rates in irrigated and non-irrigated sites were 0%. The high vector density and human feeding in irrigated sites pose major risk of malaria transmission by *An. arabiensis.* Though a mixed feeder, the species can maintain outdoor transmissions despite implementation of indoor-based interventions tools.

599

URBAN MALARIA VECTOR BIONOMICS AND POPULATION BEHAVIOR IN THREE CITIES OF SENEGAL

Abdoulaye Diop[1], Lassana Konate[2], Massila Senghor[2], Fatou Ndiaye[2], El Hadji Diouf[2], Abdoulaye Kane Dia[2], Seynabou Diedhiou[2], Ousmane Faye[2], Doudou Sene[3], Katharine Sturm-Ramirez Sturm-Ramirez[4], Sarah Zohdy[4], Ellen Dotson[4], Mame Birame Diouf[5], Lilia Gerberg[6], Abdoulaye Bangoura[7], Tiffany Clark[7], Dereje Dengela[7], Joseph Chabi[8]

[1]*Abt Associates, Dakar, Senegal,* [2]*UCAD, Dakar, Senegal,* [3]*NMCP, Dakar, Senegal,* [4]*Centers for Disease Control and Prevention (CDC), Atlanta, GA, United States,* [5]*United States Agency for International Development (USAID), Dakar, Senegal,* [6]*PMI, USAID, Dakar, WA, United States,* [7]*PMI VectorLink, Rockville, NY, United States,* [8]*PMI VectorLink, Abidjan, Côte D'Ivoire*

In recent years, a high malaria incidence has been reported in urban and peri-urban areas of Senegal. An urban landscape analysis was conducted in three cities to assess vector species composition, behavior, and density as well as transmission factors to guide the optimal deployment of vector control interventions. Entomological monitoring using human landing catches (252 person-nights), pyrethrum spray catches (420 rooms), and mosquito habitat mapping was conducted from May to December 2019 in Diourbel, Kaolack, and Touba, the most populated cities in Senegal after the capital Dakar. Additionally, a household survey was conducted in 1,202 randomly selected houses to assess house structures, sleeping spaces, sleeping behavior, and population knowledge of malaria and vector control measures. Of the 8,240 *Anopheles* mosquitoes collected in all sites, 99.4% (8,191) were *An. gambiae* s.l., specifically *An. arabiensis* (99%). The average human biting rate [14.2 bites/person/night (b/p/n)] was higher outdoors (15.9 b/p/n) than indoors (12.5 b/p/n) and average entomological inoculation rates ranged from 3.7 infectious bites per person per year (ib/p/y) in Diourbel to 40.2 ib/p/y in Kaolack. Low anthropophilic rate was recorded at all sites (average 35.7%). Of the 56 permanent larval habitats monitored during the rainy and the dry seasons, 80% (8/10) in Diourbel, 67% (12/18) in Kaolack and 43% (12/28) in Touba were productive throughout both seasons. Additionally, more than 86% of household members slept outdoors with insecticide treated nets except during the short rainy season despite having an understanding of how malaria is transmitted and the vector control measures that can prevent it. In conclusion, *An. arabiensis* is the primary malaria vector in urban Senegal with sustained permanent larval habitats found throughout the rainy and dry seasons. Since malaria vectors bite more frequently outdoors than indoors, and 86% of households sleep outdoors, these data call for complementary tools and approaches for malaria vector control.

600

CD8+ T CELL CROSS-REACTIVITY DURING HETEROLOGOUS FLAVIVIRUS INFECTION RESULTS IN CROSS-REACTIVE IMMUNODOMINATION AND ENHANCED CYTOLYTIC CAPACITY AT THE EXPENSE OF VIRUS-SPECIFIC RESPONSES

Mariah Hassert, James D. Brien, Amelia K. Pinto

Saint Louis University, St. Louis, MO, United States

Flaviviruses constitute a genus of closely related arthropod-borne viruses including Zika virus, the four serotypes of dengue virus, and yellow fever virus, which circulate in the same geographic regions. These viruses share a substantial degree of genetic similarity and consequently, antigenic overlap has been reported by evaluating T cell and antibody responses in humans, non-human primates, and mice. However, it has not been determined how existing immunity to a heterologous flavivirus impacts functional immune responses to virus-specific and cross-reactive epitopes. We hypothesize that cross-reactive T cells from prior flavivirus exposures will expand robustly during a heterologous challenge and that these cells primed during a heterologous infection will be functionally different from cells of the same specificity primed during a homologous infection. Using various mouse models of flavivirus infection, we have identified a pan-flavivirus reactive $CD8^+$ T cell epitope. We show in a heterologous infection model in which dengue virus exposure precedes Zika virus infection, T cell responses to the cross-reactive epitope dominate at the expense of the Zika-specific T cell responses. These cross-reactive T cells display enhanced killing capacity, in addition to other functional changes. The culmination of these features in heterologously primed cross-reactive T cells drive a reduction in viral burden, but enhanced immunopathology when compared to homologously primed T cells in our mouse model of Zika infection. Our findings provide a mechanistic understanding of cross-reactive T cell control during heterologous infection and have important implications for vaccine design, as these results define the functional consequences of priming a cross-reactive T cell response for a pan-flavivirus vaccine.

601

DENGUE SEROTYPE 3 WAS THE MAJOR CONTRIBUTOR IN THE RECENT EPIDEMIC IDENTIFIED FROM A TERTIARY CARE HOSPITAL OF RURAL BANGLADESH

Muntasir Alam[1], M Ishrat Jahan[1], Farzana Islam[1], Kyu Han Lee[2], Abu Pervez[3], Shafina Jahan[1], Afruna Rahman[1], Mohammed Ziaur Rahman[1], Mohammad Mamun Alam[1], Rubaiyat Yameen[1], Sanwarul Bari[1], Emily S. Gurley[2], Shams El Arifeen[1], Mustafizur Rahman[1]

[1]*icddr,b, Dhaka, Bangladesh,* [2]*Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [3]*Faridpur Medical College Hospital, Faridpur, Bangladesh*

A serosurvey conducted during 2014 -2015 showed that the distribution of dengue virus infection in Bangladesh is very heterogeneous. From 2013-2016, DENV-1 and DENV-2 serotypes were dominant and in 2017 were DENV-3. In mid-2019, a nation-wide dengue outbreak was reported. Dengue serotype data from rural Bangladesh are limited. The aim of the current study was to explore the circulating dengue serotypes in rural and central Bangladesh during 2019 outbreak. Child Health and Mortality Prevention Surveillance (CHAMPS) project is working at Faridpur Medical College Hospital (FMCH) a tertiary care health facility located in Faridpur district to understand the cause of under-5 death. A physician call center established for supporting the people living in Baliakandi, Rajbari District reported a surge in febrile illness related complaints. The objective of the current study was to identify whether the communities (including CHAMPS study area) taking health service at CHAMSP study facility were under threat of Dengue outbreak and if yes what was the serotype. From August-November 2019, a total of 66 serum samples with clinical information were available from children aged 3 months to 12 years, admitted with febrile illness at FMCH. Three children were from call center coverage area. With different set of primers by polymerase chain reaction dengue virus RNA was detected in 10 (14% of 66) serum samples. The positive patients had fever from 38.5° to 40.5° C, lethargy (40%), restlessness (40%), conjunctivitis (30%), skin rash (30%), vomiting (70%) and abdominal pain (10%). None of the dengue RNA positive patients were in shock. Sequencing data indicated that majority were serotype 3 (80%), one was serotype 2 and one was undetermined. Dominion of DENV-3 indicates possible continuation of DENV-3 outbreaks in rural areas that was seen in Dhaka in 2018. The data on circulating serotypes are necessary to understand the possible increased incidence and severity of dengue. Therefore, surveillance to identify dengue serotypes circulating in both rural and metropolitan cities are necessary for the preparation for and mitigation of future outbreaks.

603

BAYESIAN SPATIOTEMPORAL MODELING WITH SLIDING WINDOWS TO CORRECT REPORTING DELAYS FOR REAL-TIME DENGUE SURVEILLANCE IN THAILAND

Chawarat Rotejanaprasert

Mahidol University, Bangkok, Thailand

The ability to produce timely and accurate estimation of dengue cases can significantly impact disease control programs. A key challenge for dengue control in Thailand is the systematic delay in reporting at different levels in the surveillance system. Efficient and reliable surveillance and notification systems are vital to monitor health outcome trends and early detection of disease outbreaks which vary in space and time. Predicting the trend in dengue cases in real-time is a challenging task in Thailand due to a combination of factors including reporting delays. We present decision support using a spatiotemporal nowcasting model which accounts for reporting delays in a Bayesian framework with sliding windows. A case study is presented to demonstrate the proposed nowcasting method using weekly dengue surveillance data in Bangkok at district level in 2010. The overall real-time estimation accuracy was 70.69% with 59.05% and 79.59% accuracy during low and high seasons averaged across all weeks and districts. The results suggest the model was able to give a reasonable estimate of the true numbers of cases in the presence of delayed reports in the surveillance system. With sliding windows, models could also produce similar accuracy to estimation with the whole data. A persistent challenge for the statistical and epidemiological communities is to transform data into evidence-based knowledge that facilitates policy making about health improvements and disease control at the individual and population levels. Improving real-time estimation of infectious disease incidence is an important technical development. The effort in this work provides a template for nowcasting in practice to inform decision making for dengue control.

605

HIGH DENGUE BURDEN AND CIRCULATION OF ALL FOUR SEROTYPES AMONG CHILDREN WITH UNDIFFERENTIATED FEVER IN KENYA (2014-2017)

Melisa M. Shah[1], Bryson A. Ndenga[2], Francis M. Mutuku[3], David M. Vu[1], Elysse N. Grossi-Soyster[1], Victoria Okuta[2], Charles O. Ronga[2], Philip K. Chebii[4], Priscilla Maina[4], Zainab Jembe[5], Carren M. Bosire[6], Jael S. Amugongo[6], Malaya K. Sahoo[1], ChunHong Huang[1], Jenna Weber[1], Sean V. Edgerton[7], Jimmy Hortion[1], Shannon N. Bennett[7], Benjamin A. Pinsky[1], A. Desiree LaBeaud[1]

[1]*Stanford University School of Medicine, Stanford, CA, United States,* [2]*Kenya Medical Research Institute, Kisumu, Kenya,* [3]*Technical University of Mombasa, Mombasa, CA, United States,* [4]*Msambweni County Referral Hospital, Msambweni, Kenya,* [5]*Diani Health Center, Ukunda, Kenya,* [6]*Technical University of Mombasa, Mombasa, Kenya,* [7]*California Academy of Sciences, San Francisco, CA, United States*

Dengue is the most prevalent mosquito-borne viral illness worldwide. Dengue causes a broad clinical spectrum of disease, ranging from asymptomatic or mild febrile illness to hemorrhagic fever syndrome. Little is known about the epidemic and endemic circulation of dengue viruses in Africa or the predominant serotypes. This is largely due to limited point of care diagnostics and laboratory testing for DENV. Acutely ill febrile children from 1,030 outpatient visits were recruited from four clinical sites in western and coastal Kenya. Cross-sectional clinical and laboratory data were collected. Highly sensitive real-time reverse transcriptase polymerase chain reaction, genomic sequencing, and phylogenetic analyses were conducted to characterize dengue transmission among study participants between 2014-2017.Dengue viremia was detected in 41% (363/870) of children with undifferentiated febrile illness in Kenya. Of children with confirmed dengue viremia, 50% (143/286) were positive for malaria by microscopy. We sequenced dengue virus from 30 febrile participant visits. All four dengue serotypes were detected, and phylogenetic analyses revealed several viruses from novel lineages rather than from previously reported strains in Africa. We report the first dengue serotype 4 sequences from Kenya. Dengue is a major cause of fever among Kenyan children, and all four serotypes circulate with endemic and epidemic patterns. Human movement and importations of dengue from Asia to East Africa are important drivers of dengue circulation in Kenya. This study highlights the need for more consistent surveillance, robust sampling, and improved diagnostics for dengue in Africa.

606

URBANICITY DRIVERS OF DENGUE TRANSMISSION ACROSS ECUADOR, 2015-2016

Savannah N. Boerger[1], Leah C. Katzelnick[2], Josefina Coloma[2], Varsovia Cevallos[3], Emmanuelle Quentin[4], Gwenyth O. Lee[1], Joseph N. Eisenberg[1]

[1]*University of Michigan, Ann Arbor, MI, United States,* [2]*University of California, Berkeley, Berkeley, CA, United States,* [3]*Insituto Nacional de Investigación en Salud Públic, Quito, Ecuador,* [4]*Centro de Investigación en Salud Pública y Epidemiología Clínica (CISPEC), Universidad UTE, Quito, Ecuador*

Dengue was historically considered an urban disease as *Aedes aegypti* are adapted to the built human environment, yet recent research shows a

significant burden exists in rural areas. The role of urbanicity and dengue burden is not well understood, globally or within Ecuador, particularly in the context of transmission intensity at fine geographic scales. This analysis evaluated whether parish-level dengue transmission dynamics in Ecuador differ by various metrics of urbanicity, including percent "urban" residents per parish, population density, an urbanicity composite index created for this analysis, and individual urbanicity index categories. Cases of Dengue with Warning Signs (n=2405) and Severe Dengue (n=95) were reported by health professionals to a passive surveillance system in Ecuador from January 2015 to December 2016. Weighted least squares (WLS) regression models, unadjusted and adjusted for population age structure, were used to assess the relationship between the four urbanicity metrics and a proxy for force of infection: mean age of observed dengue cases. Over the study period, 231 parishes across 22 of the 24 Ecuadorian provinces in regions of plausible dengue transmission reported at least one dengue case with a mean case age of 21.6 (SD 16.5) years old. Aggregate measures of urbanicity were not significant predictors of lower mean age of dengue cases, but when considered individually, education, degree of health facility access, parish crowding, WaSH, and the labor market were significantly associated with the outcome. Urbanicity is a complex construct, but only specific factors of the urban environment were relevant to dengue transmission intensity in the Ecuadorian context. The impact of social connectivity between urban and rural areas and the influence of urbanicity on vector abundance must be assessed further. Applying these methods to surveillance data in other countries may improve our understanding of urbanicity drivers of dengue transmission, highlight the underrecognized burden of dengue in rural areas, and aid in developing more targeted dengue control strategies for both urban and rural areas.

607

ANTIBODY FC EFFECTOR FUNCTIONS AS IMMUNE CORRELATES OF PROTECTION AGAINST SYMPTOMATIC DENGUE VIRUS INFECTION

Antonio Gregorio Dias Junior[1], Carolin Loos[2], Vicky Roy[2], Caroline Atyeo[2], Sandra Bos[1], Magelda Montoya[1], Parnal Narvekar[1], Leah Katzelnick[1], Colin Warnes[1], Josefina Coloma[1], Premkumar Lakshmanane[3], Laura White[3], Angel Balmaseda[4], Aravinda de Silva[3], Galit Alter[2], Eva Harris[1]

[1]*Division of Infectious Diseases & Vaccinology, School of Public Health, University of California, Berkeley, Berkeley, CA, United States,* [2]*Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, United States,* [3]*Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States,* [4]*Laboratorio Nacional de Virología, Centro Nacional de Diagnostico y Referencia, Ministry of Health, Managua, Nicaragua*

Dengue is a mosquito-borne illness caused by one of 4 dengue virus serotypes (DENV1-4). Infections can be inapparent or present with a wide clinical spectrum. Severe cases are often associated with a heterotypic secondary (2°) infection. In our long-standing pediatric cohort study in Nicaragua, we have shown that pre-infection DENV neutralizing antibodies (Abs) play a protective role against symptomatic infection, while low pre-existing Ab titers can enhance disease severity. Further, Abs can confer protection or mediate risk through other mechanisms. The role of Ab Fc effector functions in dengue has not been systematically investigated and is a critical gap in knowledge. Here, we explored the Fc region of anti-DENV Abs as novel immune correlates. We selected pre-infection sera from 30 inapparent and 29 symptomatic 2° DENV3 infections in our cohort study and incubated these samples with DENV1-4 and ZIKV recombinant envelope protein (recE)- and non-structural protein 1 (NS1)-coupled beads. Biophysical features were assessed by Luminex. Effector functions were measured by incubating immune complexes with monocytic cells (Ab-dependent cellular phagocytosis [ADCP]), primary neutrophils (Ab-dependent neutrophil phagocytosis), primary NK cells (Ab-dependent cellular cytotoxicity [ADCC]) or complement (Ab-dependent complement deposition [ADCD]). We then looked for features that correlated with protection from subsequent DENV3 symptomatic 2° infection. We found that total IgG and IgG4 levels against NS1 and recE, respectively, and binding to the Fc receptor FcγRIIIA were higher in the pre-inapparent infection samples, indicating a potential role in protection. Our analyses also suggest protection can be mediated by ADCP, ADCC, and ADCD. Several findings were observed with recombinant antigens from different DENV serotypes, associating these features with cross-reactive Abs. Thus, we identified particular IgG isotypes, Fc receptor binding capacity and effector functions as candidate immune correlates of protection against symptomatic DENV3 secondary infection and are currently validating these findings.

608

RETHINKING DENGUE VIRUS IMMUNITY: DELAYED OR NO DETECTABLE SEROCONVERSION AFTER DENV INFECTION IN CHILDREN IN KENYA

David M. Vu[1], Elysse N. Grossi-Soyster[1], Bryson A. Ndenga[2], Francis M. Mutuku[3], Jael S. Amugongo[3], Sandra K. Musaki[2], Loice C. Lwamba[2], A. Desiree LaBeaud[1]

[1]*Stanford University School of Medicine, Stanford, CA, United States,* [2]*Kenya Medical Research Institute, Kisumu, Kenya,* [3]*Technical University of Mombasa, Mombasa, Kenya*

Presence of IgG antibody to dengue virus (DENV) is the serologic marker of infection and can have implications for predicting both protection and heightened susceptibility to disease. Recent reports of serum anti-DENV IgG in children in sub-Saharan Africa have provided evidence of ongoing DENV transmission in many countries where DENV burden was previously unknown. To investigate DENV transmission in Kenya, between 2014 and 2019, we enrolled 7509 children (median age 4.8 years, IQR 2.9-8.4) who presented with acute febrile illness to clinics in western and coastal Kenya. Blood samples were collected at enrolment and at one month follow up. At enrolment, 4.5% (297/6638) of serum samples tested had anti-DENV IgG by indirect ELISA, indicating prior DENV infection. Out of 3280 blood samples with sufficient volume for testing by RT-PCR, 424 (12.9%) were positive for DENV RNA. 408 (96.2%) PCR positive samples had no detectable anti-DENV IgG, suggesting primary DENV infection. Surprisingly, of the 274 subjects who had primary DENV infection who returned for follow up, only 4 (1.5%) developed detectable anti-DENV IgG by the convalescent visit (median interval 31 days, IQR 28-36). A secondary method using a commercial ELISA kit (InBios DENV *Detect*) confirmed the DENV IgG results with 96% concordance. Total serum IgG concentrations were within normal limits. Linear regression models identified no effect of age, length of follow up interval, or geographic location on odds of seroconversion. The low seroconversion rate suggests that the serum IgG response to DENV infection in Kenyan children may be muted or transitory. Reasons underlying this observed hyporesponsiveness to DENV infection are presently unknown but may be related to factors such as nutrient insecurity, or co-infections with malaria or HIV. Further research is required to investigate the extent of and potential mechanisms responsible for this phenomenon, which may have important implications on individual health outcomes and vaccine strategies. The findings raise an alert that measurement of serum antibody alone may substantially underestimate the burden of DENV infection.

609

INDIVIDUAL, HOUSEHOLD, AND ENVIRONMENTAL PREDICTORS OF SYMPTOMATIC DENGUE INFECTION IN A PERI-URBAN AREA OF CAMBODIA: A GEOSTATISTICAL ANALYSIS

Daniel M. Parker[1], Brook Jeang[1], Dara Kong[2], Somnang Man[3], Sokunthea Sreng[3], Rekol Huy[3], Rithea Leang[3], Jennifer A. Bohl[2], Jesus G. Valenzuela[2], Fabiano Oliveira[2], Jessica E. Manning[2]

[1]*University of California, Irvine, Irvine, CA, United States,* [2]*Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, Phnom Penh, Cambodia,* [3]*National Center of Parasitology, Entomology, and Malaria Control, Phnom Penh, Cambodia*

Dengue fever is a major threat to public health in Cambodia. In 2019 the nation experienced the worst recorded dengue outbreak in its history. There have been several fine-scale spatial and temporal analyses of dengue fever in Southeast Asia, the majority have focused on Thailand, but more information is needed in other nations with high dengue burdens. Here we present the results from a detailed spatial and temporal analysis of DENV infections and potential risk factors from an endemic peri-urban setting in Kampong Speu Province, Cambodia. In 2018, we began a pediatric cohort study (PAGODAS: Pediatric Assessment Group Of Dengue and Aedes Saliva in Cambodia) among children aged 2 - 9 that is set to run through the rainy season of 2021. Children recruited through the study (n=771) were screened twice yearly (wet and dry season) for antibodies against DENV and *Aedes aegypti* salivary gland protein extract. Symptomatic dengue infections among cohort members were monitored and recorded at a local referral hospital. Household surveys were also conducted in the target area to assess household conditions (building materials, suitability for Aedes mosquitoes, and absence/presence of larvae). The geographic coordinates (latitude and longitude) of all pediatric cohort members' residences and additionally surveyed households were recorded. Exploratory spatial analyses assessed the spatial and temporal distributions of symptomatic dengue infections in the study location. Smoothed geographic prediction surfaces were generated from household survey data and used to assess potential co-variation between neighborhood household conditions and the spatial distribution of symptomatic dengue infections. Finally, a mixed effects logistic regression was used to assess risk factors for dengue infection at multiple levels simultaneously (i.e. individual-, household-, and neighborhood-level factors). Preliminary analyses show that individual-level seropositivity to *Aedes aegypti* salivary proteins is a strong predictor of symptomatic dengue infection. This analysis will be updated with results from the 2020 rainy season by the time of presentation.

611

IMPACT OF DENGUE VIRUS GENETIC DIVERSITY ON BREADTH OF NEUTRALIZATION BY A TETRAVALENT DENGUE VACCINE

Lovkesh Karwal[1], Eduardo J. Nascimento[1], Christina DeMaso[1], Mayuri Sharma[1], Stefan Fernandez[2], Aruna Dharshan DeSilva[3], Hansi Dean[1]

[1]*Takeda Pharmaceuticals, Cambridge, MA, United States,* [2]*Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand,* [3]*Department of Paraclinical Sciences, General Sir John Kotelawala Defence University, Colombo, Sri Lanka*

Dengue viruses (DENV) are genetically diverse with different genotypes within the four DENV serotypes capable of causing disease. The virus envelope (E) plays a critical role in the virus life cycle and is the main target of neutralizing antibodies (NAb). The E protein has accumulated intra-serotype genetic diversity with heterogeneous worldwide distribution, therefore evaluation of cross-genotype immunity is essential to track vaccine coverage in different parts of the globe. Takeda's live attenuated tetravalent dengue vaccine (TAK-003) is comprised of structural proteins from each serotype in an attenuated dengue virus type 2 (DENV-2) genomic backbone. In clinical trials TAK-003 is safe and well-tolerated and elicits long-lasting NAbs against vaccine-matched DENV genotypes irrespective of baseline dengue serostatus. Our goal was to evaluate the ability of post-vaccination samples to neutralize a broader selection of virus genotypes from dengue endemic areas. A panel of genetically diverse historical (1956-2006) DENV genotypes isolated in Asia and Latin America, were tested in a microneutralization assay. Neutralization was observed across all genotypes and serotypes tested, with no significant genotype-specific differences in baseline dengue-seropositive or -seronegative vaccine recipients. The E protein amino acid sequences from contemporary DENV strains from Asia were aligned with the respective E proteins of the TAK-003 vaccine strains for each serotype. Structural modeling was used to predict location of amino acid differences with the largest potential for impact on the neutralization profile of the virus genotypes. A panel of DENV-specific reporter virus particles (RVPs) carrying E genes from contemporary strains were designed and constructed. Neutralization assays using these DENV RVPs and post-vaccination serum samples from TAK-003 Phase III clinical trials are currently underway. These tests will help evaluate the ability of TAK-003 to elicit antibodies capable of neutralizing contemporary DENV strains circulating in areas of high endemicity.

612

CORRELATION BETWEEN DENGUE AND WEATHER IN YANGON, MYANMAR FROM 2012 TO 2017

Win Zaw[1], Aung Thi[2], July Ko Ko[1], Nay Yi Yi Lin[2], Zaw Lin[2], Richard J. Maude[1]

[1]*Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand,* [2]*National Malaria Control Program, Department of Public Health, Ministry of Health & Sports, Nay Pyi Taw, Myanmar*

Dengue is among the top ten childhood diseases causing hospitalization in Myanmar and one of the hyper endemic countries defined by WHO. Recently, outbreaks frequency have increased and it is not clear to what extent changes in climate contribute to these increases in dengue incidence. Understanding this relationship in Myanmar is important for the National Dengue Control Programme to inform the possible establishment of a weather based early warning system to guide the timing and extent of interventions against outbreaks. In this study, monthly dengue incidence was correlated with weather variables at different lags. Health facility monthly dengue incidence data from 2012 to 2017 were collected from the National Dengue Control Programme, Ministry of Health and Sports. Data were cleaned and geocoded at township level. Weather data were extracted from global climate monitor, the National Centers for Environmental Information (NCEI) of the US government and the Department of Meteorology and Hydrology, Myanmar. Pearson r correlation method was used to study relationship between dengue incidence per 100000 and different weather factors. Yearly cases in Yangon Region were 1104, 4730, 2653, 4321, 2149, and 7490 and cases per 100,000 population were 15, 64, 36, 59, 29 and 102 respectively. The total number of deaths were 160 with annual case fatality rates of 0.69 and annually in Yangon Region of 0.45, 0.68, 0.83, 0.51, 0.88 and 0.80. Yangon region reported 17.7% of all cases and 24.6% of the fatalities for the whole country during the study period. The correlation results showed rainfall at zero month lag was strongly correlated (r = 0.55) with dengue incidence. Averages of the minimum temperature at one month lag (r = 0.46), mean temperature at one month lag (r = 0.47), dew point temperature at one month lag (r = 0.48), maximum temperature (r = -0.49) and absolute humidity percent (r = 0.51) at zero month lag were highest significant relations with cases incidence. Climate reliably predicted the timing of the peak in dengue incidence but not the size of the peak in Yangon Region. Further work is ongoing to assess the predictive power of combinations of variables.

614

SINGLE PASSAGING OF DENGUE CLINICAL SAMPLES FOR VIRUS ISOLATION AND AMPLIFICATION DOES NOT SIGNIFICANTLY CHANGE GENOME CONSENSUS OR FREQUENCIES OF INTRA-HOST VIRAL VARIANTS

Irina Maljkovic Berry[1], Christian K. Fung[1], Tao Li[1], Simon Pollett[1], Maria Theresa Alera[2], In-Kyu Yun[3], Jun Hang[1], Louis Macareo[2], Anon Srikiatkhachorn[2], Damon Ellison[1], Alan Rothman[4], Stefan Fernandez[2], Richard G. Jarman[1]

[1]*Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [2]*Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand,* [3]*International Vaccine Institute, Seoul, Republic of Korea,* [4]*University of Rhode Island, Kingston, RI, United States*

Intra-host nucleotide variants (iSNVs) have been increasingly used in genomic epidemiology to provide more phylogenetic resolution and reconstruct fine-scale outbreak dynamics. These analyses are usually done on direct clinical samples, but in many cases these samples may not provide enough genetic material for deep sequencing and iSNV determination due to low viral loads. Isolation of the virus from clinical samples with low passage number increases viral load, but to date, no studies have investigated how such dengue virus (DENV) isolation impacts the consensus sequence, and there is no information on the iSNV changes that this isolation might also result in. In this study, we investigate consensus and iSNV frequency differences between dengue viruses sequenced directly from clinical samples and their corresponding low-passage isolates. Twenty-five DENV1 and DENV2 positive sera specimens and their corresponding viral isolates (TS-1x1, C6/36x1 passage) were obtained from a prospective cohort study in the Philippines. These were sequenced on MiSeq with minimum nucleotide depth of coverage of 1000x, and iSNVs were detected using LoFreq. For both DENV1 and DENV2 comparisons, we found that the nucleotide call concordance (including called iSNVs with variant cutoff at 5%) between direct sera sample and its cultured virus was on average 99.99%. There was a maximum of one consensus nucleotide difference between direct sample and isolate. Interestingly, we found that iSNV frequencies were also largely preserved between the samples, with an average difference in minor variant frequency of 6.8% (95CI 3.2%) and 9.6% (95CI 2.6%) for DENV1 and DENV2, respectively. Furthermore, we found no significant differences between DENV1 and DENV2 in either the number of iSNV positions per genome, or in the difference in variant frequencies between the sample pairs (direct and isolate) (p=0.36 and p=0.13, respectively, F-test). Our results show that low-passage DENV virus isolates can be used for identification of their human-derived within-host variant populations, which are increasingly being used for precision tracking in viral transmission chains.

615

SURVEY ON NEUTRALIZING ANTIBODIES AGAINST ZIKA VIRUS 2-YEAR POST-OUTBREAK IN TWO SOUTHERN THAILAND COMMUNITIES

Theerut Densathaporn[1], Rassamee Sangthong[1], Monvaris Sakolnapa[1], Pornapat Surasombatpattana[2], Marisa Kemapunmanus[2], Promsin Masrinoul[3], Sutee Yoksan[3], Edward MeNeil[1], Virasakdi Chongsuvivatwong[1]

[1]*Epidemiology Unit, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla, Thailand,* [2]*Immunology and Virology Unit, Department of Pathology, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla, Thailand,* [3]*Center for Vaccine Development, Institute of Molecular Biosciences, Mahidol University, Salaya, Nakhon Pathom, Thailand*

In 2016-2017, there were two outbreaks of Zika virus (ZIKV) in southern Thailand. We conducted this study to assess protective immunity against this virus in the affected and nearby community in order to assess the need for future vaccine. We also studied whether natural infection of endemic flaviviruses could well protect the community. The objective included (1) to determine the prevalence of neutralizing ZIKV antibodies in the outbreak areas and test the relationship between seropositivity across and distance from the index houses, (2) to examine cross-neutralizing capacity of antibodies against ZIKV and different flavivirus strains, and (3) to identify factors associated with presence of neutralizing ZIKV antibodies. Here, 2 years after the outbreak, we enrolled (1) 18 confirmed ZIKV infected (index) cases, and samples of (2) 554 residents in outbreak areas who lived at various distance from the index cases' house (3) 190 residents of non-outbreak sub-district, and (4) 805 pregnant women in the study districts. All serum specimens underwent Plaque Reduction Neutralization Test (PRNT). Ten randomly selected ZIKV sero-positive and ten sero-negative specimens were tested for Dengue virus serotype 1-4 and Japanese Encephalitis virus antibodies using PRNT. Titre above 1:10 were considered positive. We found that 17 of the 18 index cases remained sero-positive. Zika seroprevalence [95% CI] in the two outbreak districts were 66.5% [59.1-73.9%]) and 45.6% [38.8-52.5%]). From uni-variate and multi-variate analyses, sero-positivity was independent from the distance gradient from the index houses. Elderly group, those complete less years of education, and whose house did not have plantation within 100 meters were more likely to have this neutralizing antibody. Pregnant women had 41.0% [36.1-46.0%] and 23.5% [19.4-21.9%] sero-positive rates, respectively. DENV1-4 and JEV neutralizing antibodies were present in nearly all ZIKV-positive and negative subsamples. In conclusion, both ZIKV outbreak communities had inadequate level of immunity against ZIKV, especially among pregnant women. They are still at risk for future outbreaks.

616

SEROEPIDEMIOLOGICAL STUDY OF JAPANESE ENCEPHALITIS VIRUS IN CHIANG MAI, A HIGH ENDEMIC AREA OF THAILAND

Tavitiya Sudjaritruk[1], Quanhathai Kaewpoowat[2], Chanidapa Prasarakee[3], Saowalak Sarachai[3], Anne-Frieda Taurel[4], Kamolrawee Sintupat[3], Phatraporn Assawawongprom[5], Ratchaneekorn Khampan[3], Rebecca Harris[4], Jutamad Saheng[1], Joshua Nealon[4], Sutee Yoksan[6]

[1]*Department of Pediatrics, Faculty of Medicine, Chiang Mai University; Clinical and Molecular Epidemiology of Emerging and Re-emerging Infectious Diseases Cluster, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand,* [2]*Department of Internal Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand,* [3]*Research Institute for Health Sciences, Chiang Mai University, Chiang Mai, Thailand,* [4]*Vaccine Epidemiology and Modeling Department, Sanofi Pasteur, Singapore, Singapore,* [5]*Medical Department, Sanofi Pasteur, Bangkok, Thailand,* [6]*Center for Vaccine Development, Institute of Molecular Biosciences, Mahidol University, Bangkok, Thailand*

Although routine childhood vaccination with inactivated mouse brain-derived JE vaccine (MBDV) has been implemented in Thailand for >2 decades, sporadic JE cases continue to be reported. To understand seroepidemiology of JE virus (JEV) in the population living in a high endemic area of Thailand, we conducted an age-stratified, population-based, cross-sectional study to assess the level of JEV seropositivity in Chiang Mai. Nine clusters were chosen based on administrative definition: rural (n = 3), urban (n = 3), and peri-urban (n = 3) districts. Within each cluster, participants were randomly selected from 3 different age groups: adolescents 10-20 years (n = 31); adults 21-50 years (n = 33); and older adults ≥51 years (n = 33). Plaque reduction neutralization test ($PRNT_{50}$) was performed to measure neutralizing antibodies to JEV (Beijing), and titers of ≥10 (1/dil) were considered seropositive. Of 873 participants enrolled; 46% were male and 61% had household income <500 USD/month. Only 197 participants (23%) reported having received ≥ 2 doses of MBDV, of whom 99% were among adolescent participants; and 75 (9%) reported history of dengue. JEV seropositivity was 67% (95% CI: 62-73%) among adolescents, 68% (95% CI: 62-73%) in adults, and 88% (95% CI: 84-91%) in older adults. In multivariable logistic regression analysis, living in rural or peri-urban district, household income <500 USD/month, and shorter duration since last MBDV (among those vaccinated) were

associated with JE seropositivity in adolescents, whereas living in peri-urban district and household income <500 USD/month were associated factors in adults (P <0.05). For older adults, never moved across the district and household income <500 USD/month were associated with JE seropositivity, while living in rural district showed an inverse association (P <0.05). About two-thirds of adolescents and adults, and nine-tenths of older adults demonstrated JEV seropositivity, which might be attributed in part to MBDV vaccination for adolescents, and mainly natural JEV infection in older ages. Further analyses with $PRNT_{90}$ will explore possible interactions with other flavivirus antibodies.

617

DETECTION OF ZIKA INFECTION IN A COHORT OF PREGNANT WOMEN IN KENYA, 2017-2019

Erick M. Osoro[1], Irene Inwani[2], Cyrus Mugo[2], Elizabeth Hunsperger[3], Victor Omballa[4], Dalton Wamalwa[2], Jennifer R. Verani[5], Ruth Nduati[2], John Kinuthia[6], Hafsa Jin[7], Lydia Okutoyi[8], Dufton Mwaengo[9], Brian Maugo[2], James M. Machoki[9], Nancy Otieno[4], Harriet Mirieri[1], Mufida Shabibi[10], Penina Munyua[11], Kariuki Njenga[1], Marc-Alain Widdowson[12]

[1]*Paul G. Allen School for Global Animal Health, Washington State University, Nairobi, Kenya,* [2]*Department of Pediatrics and Child Health/ Kenyatta National Hospital, University of Nairobi, Nairobi, Kenya,* [3]*Division of Global Health Protection, Centers for Disease Control and Prevention-Kenya, Nairobi, Kenya,* [4]*Center for Global Health Research, Kenya Medical Research Institute, Nairobi, Kenya,* [5]*Division of Global Health Protection, Centers for Disease Control and Prevention, Atlanta, GA, United States,* [6]*Research and Programs Department, Kenyatta National Hospital / University of Nairobi, Nairobi, Kenya,* [7]*Coast General Hospital, Mombasa, Kenya,* [8]*Department of Obstetrics and Gynecology/Kenyatta National Hospital, University of Nairobi, Nairobi, Kenya,* [9]*Institute of Tropical and Infectious Diseases, University of Nairobi, Nairobi, Kenya,* [10]*Port Reitz Hospital, Mombasa, Kenya,* [11]*Division of Global Health Protection, Centers for Disease Control and Prevention, Nairobi, Kenya,* [12]*Institute of Tropical Medicine, Antwerp, Belgium*

Zika virus (ZIKV), first discovered in East Africa, re-emerged globally in 2014 and was associated with microcephaly and other birth defects. We estimate the incidence of ZIKV infection and investigate the relationship between ZIKV infection and adverse pregnancy outcomes among pregnant women in Kenya. From 2017-2019, we recruited pregnant women aged >15 years and <28 weeks pregnant, in three health facilities in Mombasa County. Follow up was monthly with a questionnaire administered to collect sociodemographic information, environmental exposures and clinical data, and a blood sample collected for ZIKV testing. We collected urine and blood samples from participants reporting a fever or rash. We recorded pregnancy outcome and at delivery, we measured birth weight, head circumference, collected cord blood, newborn blood, and examined the newborns for birth defects. We tested sera for anti-ZIKV IgM antibodies using capture enzyme-linked immunosorbent assay (ELISA) and confirmed positives using the plaque reduction neutralization test (PRNT) for ZIKV and dengue. We collected sera and urine from participants reporting fever or rash for ZIKV RNA testing by polymerase chain reaction. Among 2889 pregnant women screened, 2312 (80%) were enrolled with a mean gestational age of 19.2 weeks (SD 5.8). Of 1916 recorded deliveries, 1816 (94.6%) were live births, 66 (3.4%) stillbirths and 34 (1.8%) abortions (<22 weeks gestation). Among 1,432 deliveries with birth weight data, 83 (5.8%) had low birth weight (<2500g). All (308) samples tested by PCR were negative. Of 2,293 participants, we collected a median of 4 (IQR 2-5) sera samples per participant, and 166 (7.2%) participants were positive for Zika IgM. Of 136 (81.2%) participants with complete PRNT results, 3 (2.2%) were ZIKV positive and 18 (13.2%) dengue positive. Neither microcephaly nor neural defects were reported from the cohort. No adverse pregnancy or neonatal outcomes were reported in the 3 ZIKV positive participants. We found a very low incidence of ZIKV infection in pregnant women in Kenya. The high proportion of negative PRNT results, suggests that preexisting immunity to ZIKV is low.

618

IGG ANTIBODY DEPLETION PRIOR TO NEUTRALIZATION RESULTS IN DECREASED ANTIBODY CROSS-REACTIVITY IN SECONDARY ZIKV INFECTIONS

Sierra Mikula, Amanda Calvert, Karen Boroughs, **Claire Huang**

Centers for Disease Control and Prevention, Fort Collins, CO, United States

In 2015, Zika virus (ZIKV) began to emerge as a global health concern due to the risk of birth defects if infection of the woman occurs during pregnancy. Given that the virus has rapidly spread through the Americas, definitive serodiagnosis of ZIKV is imperative particularly for pregnant women. Closely related flaviviruses, dengue viruses 1-4 (DENV 1-4), share a similar mosquito vector, global distribution, and symptomology as ZIKV. DENVs also elicit long-lasting antibodies that are cross-reactive to ZIKV, making differential serodiagnosis between ZIKV and DENV infection difficult. Here, we investigate ZIKV-specific neutralizing IgM antibody responses from individuals with ZIKV infections and previous exposure to DENV using longitudinal diagnostic specimens [0-500 days post-index (PI)] in a reporter microfocus reduction neutralization test with 90% endpoint cutoff (R-$mFRNT_{90}$). When neutralization endpoint titers were compared pre- and post- IgG depletion in samples, cross-reactive neutralizing antibodies were significantly reduced in 62.9% of the 35 samples tested. These results contribute to our understanding of IgM neutralization and show the potential of this assay to aid in distinguishing flaviviral infections using serodiagnositcs.

619

PREVENTION OF SEXUAL AND VERTICAL TRANSMISSION OF ZIKA VIRUS (ZIKV) FOLLOWING IMMUNIZATION WITH A LIVE-ATTENUATED ZIKV VACCINE CANDIDATE

Janae Stovall[1], Whitney Baldwin[2], Holli Giebler[2], Hansi Dean[3], Jill Livengood[2], Claire Huang[1]

[1]*U.S. Centers for Disease Control and Prevention, Fort Collins, CO, United States,* [2]*Takeda Vaccines; CDC/NCEZID/DVBD/ADB, Cambridge, MA, United States,* [3]*Takeda Vaccines, Cambridge, MA, United States*

The declaration of a public health emergency by the WHO as a result of the South and Central American Zika virus (ZIKV) outbreak in 2015-2016 has brought the need for a vaccine to protect against ZIKV-induced disease, including congenital Zika syndrome, to the forefront. Towards this goal, we introduced the prM and E genes of ZIKV into an attenuated dengue-2 (DENV2; PDK-53) vaccine virus backbone, to derive chimeric DENV2/ZIKV live-attenuated vaccine (LAV) candidates against ZIKV. In mice and non-human primates, these candidates displayed an excellent safety profile and were immunogenic and efficacious for prevention of viremia upon peripheral challenge. However, in addition to mosquito transmission, ZIKV can be sexually and vertically transmitted, necessitating additional transmission routes to consider when evaluating vaccine safety and efficacy. Using the AG129 mouse model, we evaluated the safety and efficacy of our LAV candidates regarding both sexual and vertical transmission routes of ZIKV. We determined the vaccine candidates did not replicate within the male reproductive tract and were not sexually transmissible to naïve females. Additionally, a single immunization of the LAV candidates, administered 30 days prior to mating, conferred protection to subsequently pregnant AG129 females and their offspring against ZIKV infection via a sexual transmission route (immunized female mated with ZIKV-infected male) as well as a vertical transmission route (immunized female intraperitoneally challenged with ZIKV 5.5 days post-mating). In summary, these results provide additional safety and protective efficacy profiles of the chimeric DENV2/ZIKV LAV candidates relating to sexual transmission, pregnancy and congenital disease in a highly susceptible mouse model.

620

NEUROLOGICAL SEQUELAE OF ACQUIRED ZIKA VIRUS INFECTION AMONG CHILDREN IN MANAGUA, NICARAGUA

Jill F. Lebov[1], Anna Gajewski[2], Oscar Ortega[2], Guillermina Kuan[3], Eva Harris[4], Emma Crenshaw[1], Stephen R. Hooper[5]

[1]*RTI International, Research Triangle Park, NC, United States,* [2]*Sustainable Sciences Institute, Managua, Nicaragua,* [3]*Health Center Socrates Flores Vivas, Managua, Nicaragua,* [4]*Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, Berkeley, CA, United States,* [5]*Department of Allied Health Sciences, School of Medicine, University of North Carolina, Chapel Hill, Chapel Hill, NC, United States*

Although many studies have evaluated the biological pathways for congenital Zika virus (ZIKV) infection and severe neurodevelopmental outcomes, research into child health outcomes related to postnatally acquired ZIKV infection is limited. Most epidemiological studies include probable cases based on signs and symptoms rather than confirmed cases. Case reports and surveillance of acute central nervous system manifestations suggest that ZIKV may have a wider impact on neurological health beyond that observed in congenitally exposed newborns, including neuropsychological deficits, long-term fatigue, and spinal cord involvement (e.g., paresthesia and muscle weakness). To better understand neurological impacts of ZIKV in children, we evaluated the incidence of neurological symptoms in children with postnatally acquired ZIKV infection in Nicaragua. Children presenting to the study health center with Zika-like symptoms were tested for ZIKV by RT-PCR and assessed for neurological symptoms at acute visits 1-5 days post-onset of symptoms and subsequent clinical visits through 1-year post-infection. Follow-up clinical neurological and neuropsychological assessments of study children were conducted 3-4 years post-infection. Of 201 ZIKV-infected children enrolled, 55% were female and the average age was 8 (min, max: 2, 13). Within 1-year post-infection, neck stiffness, seizures, muscle weakness or paralysis, and paresthesia were not observed among study children. Asthenia was observed at a single clinical visit for 3 children. Vertigo was reported for one child at 3 visits 27-39 days after ZIKV infection onset but co-occurred with a urinary tract infection diagnosis. Two other children had a single report of vertigo: one co-occurred with a cold; one had no other diagnoses present. While a direct relationship between ZIKV infection and neurological manifestations was not observed in the first year post-infection, it is possible that children with more severe neurological sequelae attended the hospital rather than the study clinic or that neuropsychological impacts are present. Results of follow-up assessments will be presented.

621

CO-CIRCULATION OF ZIKA VIRUS AND DENGUE VIRUS SEROTYPES 2 AND 3 IN GUERRERO STATE, MEXICO, 2019

Daniel Nunez Avellaneda[1], Chandra Tangudu[1], Luz Alarcon-Romero[2], Ma Isabel Zubillaga-Guerrero[2], Jacqueline Barrios-Palacios[3], Carlos Machain-Williams[4], Salatiel Nunez-Avellaneda[5], Laura Loaeza-Díaz[6], Bradley Blitvich[1]

[1]*Iowa State University, Ames, IA, United States,* [2]*Universidad Autonoma de Guerrero, Chilpancingo, Guerrero, Mexico,* [3]*National Institute of Medical Sciences and Nutrition Salvador Zubirán, Ciudad de México, Mexico,* [4]*Universidad Autónoma de Yucatán, Merida, Mexico,* [5]*Hospital General Adolfo Prieto, Taxco de Alarcon, Guerrero, Mexico,* [6]*Hospital General Raymundo Abarca Alarcón, Chilpancingo Guerrero, Mexico*

Flaviviruses have an enormous impact on human health in numerous countries, including Mexico, but limited work has been done to monitor flavivirus activity in the state of Guerrero, southwestern Mexico. The goal of this study is to assay febrile patients and mosquitoes from Guerrero in 2019 for evidence of flavivirus infection. Sera were collected from 651 patients who presented with unspecified fever at three hospitals. Additionally, 803 mosquitoes were collected by manual aspiration. To date, 410 sera have been tested for dengue virus (DENV) antigen by enzyme-linked immunosorbent assay, with 62 (15.1%) testing positive. All sera have been tested at a 1:20 dilution by plaque reduction neutralization test (PRNT) using DENV-2, with 419 (64.4%) testing positive. Sixty sera with flavivirus-specific antibodies were randomly selected, titrated and further tested by PRNT using all four DENV serotypes, St. Louis encephalitis virus, West Nile virus and Zika virus (ZIKV). Eight (13.3%) patients were seropositive for DENV-2, four (6.7%) patients were seropositive for DENV-3, six (10%) patients were seropositive for ZIKV, 18 (30%) patients had secondary flavivirus infections and 24 (40%) patients had antibodies to an undetermined flavivirus. The entomologic investigation yielded 736 *Aedes aegypti* and 94 *Culex quinquefasciatus* that were sorted into 183 pools and 20 pools, respectively. All mosquitoes were assayed for flavivirus RNA by RT-PCR and Sanger sequencing. DENV2 sequence detected in three pools of *Ae. aegypti*. In summary, we provide evidence of concurrent circulation of DENV-2, DENV-3 and ZIKV in Guerrero, Mexico.

622

CYNOMOLGUS MACAQUES ARE RESISTANT TO SPONDWENI VIRUS INFECTION

Anna S. Jaeger[1], Chelsea M. Crooks[2], Andrea M. Weiler[2], Mason I. Bliss[2], Sierra L. Rybarczyk[2], Eric Peterson[3], Saverio Capuano[4], Thomas C. Friedrich[2], Matthew T. Aliota[1]

[1]*University of Minnesota, Saint Paul, MN, United States,* [2]*University of Wisconsin-Madison, Madison, WI, United States,* [3]*Wisconsin National Primate Research Center, Madison, WI, United States,* [4]*Wisconsin National Primate Research Center, Madison, MN, United States*

Spondweni virus (SPONV) is the closest known relative of Zika virus (ZIKV). In 2016, SPONV was identified outside its endemic range of Africa, in a pool of Culex quinquefasciatus mosquitoes in Haiti. Recently, we demonstrated that SPONV has similar pathogenic traits to ZIKV in a mouse model of vertical transmission, and that *Aedes aegypti* mosquitoes were capable of transmitting SPONV. To further investigate SPONV pathogenesis in a translational model, we conducted a pilot study to develop a non-human primate model of SPONV infection. For this study, five cynomolgus macaques were inoculated subcutaneously (s.c.) with 10^4 PFU of the low-passage SPONV isolate SAAr94 and four animals were s.c.-inoculated with 10^4 PFU of ZIKV strain DAK AR41524. Plasma, oral swabs, and urine were collected daily to measure vRNA kinetics by QRT-PCR. Only two of five animals inoculated with SPONV had detectable plasma viremia, whereas all four ZIKV-inoculated animals were productively infected. Viral loads in animals inoculated with SPONV were much lower in magnitude and shorter in duration compared to ZIKV-infected animals. In addition, SPONV-inoculated animals failed to mount a neutralizing antibody response at 28 days post inoculation as measured by PRNT. We then challenged both cohorts of animals with a higher dose of SPONV (10^6 PFU) delivered s.c., eight weeks after initial SPONV or ZIKV exposure. A single animal previously infected with SPONV had low, but detectable, viremia lasting two days after homologous rechallenge, and two animals previously infected with ZIKV had similarly low-level viremia for 1-2 days. Additional data will be forthcoming after a pause in research due to COVID-19. Preliminary results suggest cynomolgus macaques have a degree of inherent resistance to SPONV infection. The fact that macaques show such different levels of susceptibility to two such closely related viruses opens the possibility for defining novel flavivirus host restriction factors and mechanisms of innate immune evasion, and may aid in the development of more appropriate animal models for investigating flavivirus pathogenesis.

623

SPATIAL AND SPATIOTEMPORAL INSIGHTS FROM SEQUENTIAL CHIKUNGUNYA AND ZIKA EPIDEMICS IN A PEDIATRIC COHORT IN MANAGUA, NICARAGUA

Fausto A. Bustos Carrillo[1], Brenda L. Mercardo[2], Jairo C. Monterrey[2], Damaris Collado[2], Tatiana Miranda[2], Sergio Ojeda[2], Nery Sanchez[2], Miguel Plazaola[2], Harold Laguna[2], Maria M. Lopez[2], Douglas Elizondo[2], Sonia Arguello[2], Saira Saborio[2], Josefina Coloma[1], Leah Katzelnick[1], Aubree Gordon[3], Hugh Sturrock[4], Angel Balmaseda[5], Guillermina Kuan[6], Eva Harris[1]

[1]*Division of Infectious Diseases & Vaccinology, School of Public Health, University of California, Berkeley, Berkeley, CA, United States,* [2]*Sustainable Sciences Institute, Managua, Nicaragua,* [3]*Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, Ann Arbor, MI, United States,* [4]*Department of Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, CA, United States,* [5]*Laboratorio Nacional de Virología, Centro Nacional de Diagnóstico y Referencia, Ministry of Health, Managua, Nicaragua,* [6]*Centro de Salud Sócrates Flores Vivas, Ministry of Health, Managua, Nicaragua*

Chikungunya virus (CHIKV) and Zika virus (ZIKV), arboviruses transmitted by Aedes mosquitoes, recently spread across the Americas. Households are thought to serve as the site of transmission, leading to household-based interventions. Further, the typical use of cases (symptomatic infections) as the basis for spatial studies conflates the separate infection and disease processes. Here, we characterized the spatial and spatiotemporal epidemiology of two chikungunya (2014-2015) and one Zika (2016) epidemic in the Pediatric Cohort Dengue Study, an ongoing prospective study of 2-14-year-olds in Managua, Nicaragua. Around 3,000 initially CHIKV- and ZIKV-naïve PDCS participants were analyzed for each epidemic. Infections were confirmed by inhibition and competition ELISAs, and cases were confirmed by rRT-PCR and serological assays. All analyses georeferenced participants to their household GPS points. Kuldorff's spatial scan test, spatiotemporal generalized additive models, and mixed-effects geostatistical models were used to analyze the data. Surprisingly, across the 3 epidemics, infection and disease outcomes were not correlated within households. Rather, infections were spatially correlated across distances <50m. During the large 2015-2016 epidemics, a seroprevalence gradient ranging from >60% around a cemetery abutting the study area to the west to <35% in the northeastern region was observed. The spatial pattern of infections was mostly driven by distance to the cemetery, as the gradient was lost upon conditioning on distance to the cemetery in a geostatistical model. Northern and eastern study areas had clusters of non-infected persons, suggesting that residents there remain at risk. While large clusters of infections were identified, no clusters of disease were found after conditioning on infection status, suggesting that only the infection process is spatially mediated. The focal point of disease shifted in location and space differently across each epidemic. Overall, these results help inform public health interventions targeting the built environment and future modeling of arboviral transmission dynamics.

624

ZIKA VIRUS RECRUDESCENCE IN THE MURINE MALE REPRODUCTIVE TRACT FOLLOWING IMMUNOSUPPRESSION

Megan B. Vogt, Nisha K. Duggal

Virginia Polytechnic Institute and State University, Blacksburg, VA, United States

Zika virus (ZIKV; *Flaviviridae*, *Flavivirus*) is an emerging arbovirus that typically causes a mild febrile illness in adults but may cause congenital malformations in infants infected *in utero*. During the recent ZIKV epidemic, male-to-female ZIKV sexual transmission was reported, and ZIKV sexual transmission to pregnant women was shown to result in congenital ZIKV syndrome in infants. Infectious ZIKV and ZIKV RNA can be shed in semen of infected men up to thirty days and six months post disease onset, respectively. Currently, it is unknown whether prolonged ZIKV RNA in semen represents persistent infection in the male reproductive tract (MRT), which may allow ZIKV to recrudesce after the initial period of infectious virus shedding. Here, we investigated whether ZIKV recrudesces in the MRT following immunosuppression in a mouse model. In this study, C57BL6/J male mice were pre-treated with a blocking antibody against interferon α/β receptor (IFNAR1) and infected subcutaneously with ZIKV. When mice no longer shed infectious ZIKV in ejaculates, they were treated with one of the following immunosuppressants, chosen for differing mechanisms of action: anti-IFNAR1 antibody, cyclophosphamide, cyclosporine/ketoconazole, dexamethasone, or methylprednisolone acetate. Following immunosuppression, no clinical signs of ZIKV infection or viremia were observed. Infectious ZIKV was not detected in ejaculates and rarely detected in MRT tissues of mice following immunosuppression. ZIKV RNA was observed in ejaculates of mice throughout the study; analysis is ongoing as to whether ZIKV RNA in ejaculates increased post-immunosuppression. ZIKV RNA was present in epididymides of most mice following immunosuppression, with significantly higher RNA levels in mice treated with cyclophosphamide compared to PBS control. Overall, our results do not eliminate the possibility of ZIKV recrudescence in the MRT. This study provides significant insight into virus persistence dynamics in the MRT and the risk of ZIKV sexual transmission. Future studies will assess the role of the epididymis in ZIKV persistence in the MRT.

625

VECTOR COMPETENCE OF HUMAN BITING TICKS *IXODES SCAPULARIS*, *AMBLYOMMA AMERICANUM* AND *DERMACENTOR VARIAIBILLIS* FOR POWASSAN VIRUS

Rohit Sharma, Duncan W. Cozens, Philip M. Armstrong, Douglas E. Brackney

The Connecticut Agricultural Experiment Station, New Haven, CT, United States

About 95% of annually reported vector-borne diseases in the United states are caused by ticks which constitute a considerable impact on the public health systems. Powassan virus lineage II (POWV-II) aka Deer tick virus), a flavivirus, is transmitted to human through the bite of infected *Ixodes scapularis* ticks (Black legged tick) which can result in neurodegenerative disease and death. Although, such infections are sporadic, the increase in the number of cases in recent years is concerning, given that a vaccine or therapeutic interventions are not available. Moreover, like other arboviruses, inherent genetic plasticity of POWV-II may enable them to efficiently adapt to a newer tick species leading to their host expansion. The dog tick, *Dermacentor varibillis* and lone star tick, *Amblyomma americanum* are known human biting ticks and widely distributed in the eastern United States, thus, it is important to determine whether these ticks can also serve as vector for POWV. As POWV has previously been isolated from field collected *Dermacentor andersoni* and *Haemaphysalis longicornis*. Therefore, we performed an experiment to assess the competency of *I. scapularis*, *D. varibillis* and *A. americanum for POWV-II.* We demonstrated that all three tick species were capable of acquiring POWV-II via feeding on a viremic mice at the larval stage, maintain infection through molting, and successively transmit it to the naïve mice at comparable rates across all three species. Data from these experiments will be presented.

626

MODELLING A ZIKA VIRUS OUTBREAK IN BRAZIL UNDER CURRENT AND FUTURE CLIMATE

Tara Sadeghieh[1], Jan Sargeant[1], Amy Greer[1], Olaf Berke[1], Victoria Ng[2]

[1]*University of Guelph, Guelph, ON, Canada,* [2]*Public Health Agency of Canada, Guelph, ON, Canada*

Zika virus (ZIKV) is primarily transmitted by *Aedes* mosquitoes between humans and non-human primates. Mosquitoes are expected to proliferate faster and spread pathogens to new locations due to climate change. The purpose of this modelling study was to determine how an outbreak similar to the 2016 ZIKV outbreak in Brazil might unfold under climate change

scenarios. A compartmental infectious disease model was developed to fit the 2016 ZIKV outbreak data from Brazil using least squares optimization, and included compartments for humans and mosquitoes. In order to explore the impact of climate change on temperature-sensitive mosquito parameters, these parameters were set to change over the projected time periods using polynomial equations fit to their relationship with current temperature, and then adjusted for the average temperature across years 2011 - 2040, 2041 - 2070, and 2071 - 2100 for Representative Concentration Pathways (RCP) 4.5 and 8.5. Climate change scenarios impacted the model outcomes, including the peak case count, cumulative cases, time to peak incidence, and the duration of the outbreak. Overall, there were non-linear changes in the outcomes, where there was an increase between the short- and medium-term time periods for both RCP scenarios, and then between the medium- and long-term there was a slight increase for RCP 4.5 and slight decrease for RCP 8.5. For RCP 4.5, between now and 2070 - 2100, the peak case count increased from 10,473 to 22,030; the time to the peak increased from 9 to 12 weeks; and the duration of the outbreak increased from 41 to 52 weeks. For RCP 8.5, the peak case count increased to 21,786; the time to the peak increased by 11 weeks; and, the duration of the outbreak increased to 50 weeks. Outbreaks of ZIKV in Brazil are expected to be more intense due to climate change. As the impacts of climate change becoming increasingly apparent on human health, it is important to quantify how large this change may be, and to determine the most effective interventions strategies.

627

SURVEILLANCE FOR ARTHROPOD-ASSOCIATED VIRUSES IN NORTHWESTERN AND SOUTHWESTERN MEXICO USING TRADITIONAL VIRUS DETECTION TECHNIQUES AND METAGENOMICS

S. Viridiana Laredo-Tiscareno[1], Javier A. Garza-Hernandez[2], Carlos Arturo Rodríguez-Alarcón[2], Diana Marcela Beristain-Ruiz[2], Jaime Raúl Adame-Gallegos[3], Rodolfo González-Peña[3], David Baylon-Jaquez[4], Ignacio Netzahualcoyotl Barajas-López[5], Adriana Camacho Perea[5], Carolina Montelongo-Ponce[2], Ramón Rivera-Barreno[2], Chandra S. Tangudu[1], Bradley J. Blitvich[1]

[1]*Iowa State University, Ames, IA, United States,* [2]*Universidad Autonoma de Ciudad Juarez, Ciudad Juarez, Mexico,* [3]*Universidad Autonoma de Chihuahua, Chihuahua, Mexico,* [4]*Secretaría de Desarrollo Urbano y Ecologia, Ciudad Juarez, Mexico,* [5]*Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Mexico*

The objectives of this study are to increase our understanding of the arthropod-associated viruses circulating in Mexico by serologically assaying domestic animals for evidence of flavivirus infection and testing mosquitoes and ticks for novel and recognized viruses by virus isolation in cell culture and metagenomics. Sera and arthropods were collected in 2018 and 2019 in the states of Chihuahua and Michoacan in northwestern and southwestern Mexico, respectively. Sera were collected from 648 domestic animals (horses, poultry, sheep, cows, dogs, cats, pigs, and rabbits). A total of 1337 mosquitoes of three genera (*Aedes, Culex,* and *Mansonia*) were also collected by manual aspiration. To date, sera from 194 horses have been tested by plaque reduction neutralization test (PRNT) using West Nile virus (WNV) and St. Louis encephalitis virus (SLEV). Of these, 113 (56.7%) horses were seropositive for WNV, three (15.3%) horse was seropositive for SLEV, 11 (5.6%) horses had antibodies to an undetermined flavivirus and 64 (32.6%) horses were negative for antibodies to flaviviruses. The mosquitoes were sorted in 35 pools, homogenized and inoculated onto monolayers of *Aedes albopictus* (C6/36) and/or *Culex tarsalis* (CT) cells. A second blind passage was performed then total RNA was extracted and analyzed by RNA-seq. Our metagenomics data are currently being analyzed for viral sequences. However, because 16 homogenates caused cytopathic effect, we consider it likely that viruses have been recovered. Ticks are currently being processed. Taken together, our ongoing serological investigation and virus discovery experiments will increase our understanding of the arthropod-borne viruses present in northwestern and southwestern Mexico.

628

WEST NILE VIRUS MUTATIONS DERIVED FROM NATURALLY OCCURRING QUASISPECIES HAVE INCREASED HOST-SPECIFIC FITNESS

Haley S. Caldwell[1], Lili Kuo[2], Sean M. Bialosuknia[2], Laura D. Kramer[2], Alexander T. Ciota[2]

[1]*State University of New York at Albany, Albany, NY, United States,* [2]*Arbovirus Laboratory, Wadsworth Center, New York State Department of Health, Albany, NY, United States*

The composition and breadth of the West Nile virus (WNV; Flaviviridae; Flavivirus) mutant swarm varied significantly between avian and mosquito hosts, presumably due to unique selective and stochastic pressures. Previous studies identified minority variants with amino acid substitutions in the WNV replicase (NS3/NS5) with host biases and a putative role in host-specific fitness. In order to better characterize the largely unknown role of the helicase in host-specific fitness, we engineered two non-synonymous NS3 mutations, P1825L (mosquito-biased) and S1852T (avian-biased) into a WNV 02 infectious clone and characterized these variants *in vitro* and *in vivo*. *In vitro* growth kinetics revealed that S1852T had similar growth kinetics to wildtype (WT) WNV in avian (DF1), mosquito (C636), and mammalian cell lines (Vero, A549) whereas P1825L had attenuated growth in vertebrate and avian cell lines, suggesting that it may be associated with host specific fitness differences. One-step growth kinetics and plaque measurements also supported attenuation of P1825L in vertebrate cells. In addition, both NS3 mutants demonstrated altered susceptibly to the mutagen ribavirin, consistent with differences in replicase fidelity and/or function. Structural modeling of P1825L revealed it was located in a highly conserved motif within the linker region between the N and C core domain of NS3, also indicating the potential for altered enzymatic activity. *In vivo* studies in *Culex pipiens pipiens* demonstrated increased infection of P1825L relative to the WT in mosquitoes, indicating a mosquito-specific advantage. In survival studies, both mutant-exposed mosquitoes had increased survival relative to the WNV WT. Interestingly, S1852P was also more infectious in mosquitoes relative to the WT despite its avian bias, yet decreased dissemination and transmission rates at later time points were also measured, potentially indicated a fitness cost in mosquitoes. Together, these results indicate that naturally occurring mutations with host biases play a role in host-specific fitness and foster host-specific adaptation.

629

SPECIES AND STRAIN-DEPENDENT EFFECTS OF TEMPERATURE ON FLAVIVIRUS ADAPTATION AND FITNESS

Rachel L. Fay[1], Kiet A. Ngo[2], Sean M. Bialosuknia[2], Laura D. Kramer[2], Alexander T. Ciota[2]

[1]*State University of New York Albany, Albany, NY, United States,* [2]*Wadsworth Center, New York State Department of Health, Slingerlands, NY, United States*

Arthropod-borne viruses are associated with over 140 human diseases. The most common arboviruses vectored by mosquitoes are those which belong to the *Flaviviridae* family, including West Nile virus (WNV), Zika virus (ZIKV) and dengue virus. Global temperatures are increasing, which directly impacts arboviruses vectored by ectothermic organisms. Increased temperatures have been shown to have a significant effect on RNA viral replication and vector competence in mosquitoes, but the impact of temperature on arbovirus evolutionary trajectories and fitness landscapes has yet to be studied. To test the hypothesis that temperature impacts the rate and extent of flavivirus evolution in mosquitoes, we experimentally passaged WNV and ZIKV 12 times in *Culex tarsalis* and *Aedes albopictus* cells, at 25C and 30C. Our results suggest that increased temperatures accelerate adaptation for both WNV and ZIKV yet also that temperature sensitivity is species specific. In addition, to test the hypothesis that variability in temperature sensitivity exists in naturally occurring strains we evaluated replicative kinetics *in vitro* and vector competence in *Culex pipiens* mosquitoes using representative strains of WNV02 and

NY10 genotypes at 25C and 30C. These data demonstrated that NY10 genotype strains are more infectious than WNV02 strains in *Cx. pipiens*, yet the extent of this advantage is both temperature and time dependent. Together, these data indicate that temperature significantly affects the adaptability and fitness of flaviviruses and that the relationship between temperature and viral fitness is dependent on both viral species and strain.

630

THE ERA OF PLANETARY CHANGE: EXTREME WEATHER EVENTS IN GEORGIA, USA, AND IMPACT ON ARBOVIRAL DISEASE EPIDEMIOLOGY

Julia Schiff[1], Uriel Kitron[2], Thomas R. Gillespie[2], Colleen S. Kraft[1], Erik A. Brownsford[1], Morgan A. Lane[1], Jessica K. Fairley[1]

[1]*Emory University School of Medicine, Atlanta, GA, United States,* [2]*Emory University, Atlanta, GA, United States*

Extreme weather events have severe impacts on infrastructure and healthcare globally with infections occurring more frequently and rare infections becoming more apparent. Our aim was to analyze the association of three recent hurricanes in Georgia, USA, - Matthew (2016), Irma (2017), and Michael (2018) - with arboviral epidemiology to promote better understanding of the impact of planetary change on risk of these infections. We analyzed county-level cases of West Nile virus (WNV), Eastern Equine Encephalitis virus (EEE), St. Louis Encephalitis virus (SLE), Jamestown Canyon virus (JTC), and LaCrosse virus (LaC) from the CDC ArboNET Database and the GA Department of Public Health from 2009-2019. We report county-level incidence, and use levels of FEMA assistance as a measure of hurricane severity. Overall incidence of WNV was highest in 2012, 2017, and 2018 (0.86, 0.47, 0.35 cases per 100,000, respectively), with cases double or triple those in other years. Notably, there was one case each of SLE and JTC in 2018, and 1-2 cases of EEE and LaC were reported in most years. Further, while county level incidence of total arboviruses from 2016-2019 was not associated with FEMA requests post hurricanes, southern counties exhibited statistically significantly higher mean incidence compared to other counties in 2017 and 2018. The highest mean annual arboviral incidence between the 2016-2019 hurricanes occurred in 2 counties located in the southwest of the state (8.16 - 9.95 / 100,000). Although numbers are low overall, results do suggest increased arbovirus in recent years, particularly in southwest and central areas most impacted by extreme weather events. Further study will include spatial analyses of arboviruses and correlation to state zoonotic infection. These findings suggest the need for greater research and resources directed towards post-natural disaster needs and other climate-related health issues.

631

VIRAL METAGENOMICS INVESTIGATION OF PATIENTS WITH FEVER OF UNKNOWN ORIGIN AND POTENTIAL INSECT VECTORS IN MAIDUGURI, NIGERIA

Bamidele Soji Oderinde[1], Olajide Olubunmi Agbede[2], Zhengli Shi[3], Saka Saheed Baba[4], Marycelin Baba[1]

[1]*University of Maiduguri, Nigeria, Maiduguri, Nigeria,* [2]*University of Ilorin, Nigeria, Ilorin, Nigeria,* [3]*Wuhan Institute of Virology, Chinese Academy of Science, Wuhan, China,* [4]*University of Ilorin,Nigeria, ilorin, Nigeria*

In a one-health approach, deep sequencing of human serum, mosquitoes and ticks provides an opportunity to monitor arboviral infections in febrile illness of unknown origin (FUO) in Maiduguri, Nigeria. Self-administered structured questionnaire containing socio demographic information of 75 patients with FUO and 75 apparently healthy blood donors as controls that are within 80 km radius of the health facility. A metagenomics analysis of purified viral particles in Human serum of healthy and FUO patients, pooled mosquitoes according to species - (Aedes58 + Anopheles 262 species, and Culex 1718) collected by baiting in dry ice and ticks by plucking 720 (Amblyomma 86, Boophilus 254 and Hyalomma 380) from the immediate environment of the patient and ticks from closest or neighboring domestic animals namely; cow, camel and sheep. The mosquitoes and ticks were collected in dry season and raining season. Results revealed sequences related to 22 eukaryotic viral families (BLASTx and BLASTn E score,$<10^{-5}$) with a minimum hit length of 20 amino acids, including known pathogens in numerous viral families infecting humans/vertebrates, fishes and amphibians, insects and archae / fungi / bacteria. Sequences of *Flaviridae* and *Herpesvidae* are prominent in patients with FUO and all the vectors. Interestingly, 17 discrete families of virus sequences detected in pooled sera of patients with FUO; seven were RNA and 10 DNA viral families, inclusive of insect variants viruses detected. However, insect and tick variants of viral sequences have small genomic sizes which may have evolutionary significance and could have introduced diversity into the ecology of viruses, apparently, the larger the genomic size, the lesser the possibility that the variant of the virus will exist in insects. The large genomic sizes carried by most DNA viruses cannot probably be propagated in insects and ticks but they can, presumably, be transmitted to mammals including humans. There is 90.9% inter transmission rate in the environment between humans, animals, insects and ticks. The overall study has implications for surveillance of emerging viruses and prediction of viral epidemics.

634

IMPACT OF HURRICANE IRMA ON ARBOVIRAL TRANSMISSION IN FLORIDA

Miguel Reina Ortiz, Ismael Hoare, Daniel Chacreton, Desiree Lara, Ricardo Izurieta

University of South Florida, Tampa, FL, United States

Rainfall and windspeed have been associated with vector-borne disease transmission in non-disaster contexts. Data evaluating the impact of hurricanes on arboviral transmission is limited. This study evaluates the impact of Hurricane Irma on arboviral transmission in Florida in 2017, traditionally surveilled by sentinel chicken seroconversion. West Nile virus (WNV) and Eastern Equine Encephalitis virus (EEEV) sentinel chicken seroconversion data spanning pre- and post-hurricane periods were collected from the Florida Department of Health. A zero-inflated negative binomial regression was used to assess the impact of Hurricane Irma's rain and wind on the number of positive chickens per week in the post-hurricane period, controlling for weekly meteorological data (wind, rain, temperature), 10-year seasonal variation, and distance to the eye of the storm. The difference between post- and pre-hurricane cumulative incidence was modeled by a stepwise backward linear regression, controlling for the same variables. Irma rainfall was negatively associated with post-hurricane weekly seropositivity ($\beta = -0.74$, $p < 0.0001$) whereas weekly wind speed had a positive effect ($\beta = 0.19$, $p = 0.0243$). Increased distance to the storm eye tends to correlate with lower seroconversion ($p = 0.1845$). For EEEV, post-hurricane transmission data was scarce and a model could not be fit. Irma rainfall ($\beta = -1.09$, $p = 0.013$) and wind ($\beta = 0.53$, $p = 0.001$) were associated with EEEV post-pre hurricane seroconversion difference whereas no effect of Hurricane Irma's meteorological characteristics on WNV pre-post seroconversion difference could be observed. In conclusion, hurricanes may have differential effect on arboviral transmission depending on rainfall and wind speed. Hurricane Irma's rainfall had negative whereas hurricane wind speed had positive effects on WNV (rain only) and EEEV (both) transmission in Florida. Further research is needed to elucidate the intricate relationship between the geographic and meteorological characteristics of a hurricane and its effect on arboviral disease transmission.

635

SEROPREVALENCE AND RISK FACTORS RELATED TO LASSA FEVER IN SIERRA LEONE

Emily J. Engel[1], Lansana Kanneh[2], Jeffrey G. Shaffer[3], Lina Moses[3], Donald Grant[2], Robert F. Garry[4], John S. Schieffelin[4]

[1]*Tulane University, New Orleans, LA, United States,* [2]*Kenema Government Hospital, Kenema, Sierra Leone,* [3]*Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States,* [4]*Tulane University School of Medicine, New Orleans, LA, United States*

Lassa Fever (LF) is an acute viral hemorrhagic illness caused by the Lassa virus (LASV). Infections in humans occur after exposure to household items, foodstuffs, or water that has been contaminated by the rodent-host *Mastomys*. Very little is known about the true seroprevalence of LF in endemic countries, including Sierra Leone. A large-scale study, in partnership with Kenema Government Hospital's (KGH) Lassa Fever Outreach Team, was conducted in three districts of Sierra Leone – Kenema (LF endemic), Tonkolili (LF emerging), and Port Loko (non-endemic for LF). In this study, we determined the point seroprevalence of LF and potential risk factors for LASV seropositivity in this region.Subjects were selected using a two-stage cluster sampling method in which 25 to 30 villages per district and 12 households per village were randomly selected. Blood was collected via finger stick from up to twelve members of each household. Over 11,000 dried blood spots (DBS) were collected in total. These DBS were tested for LASV nucleoprotein (NP) antibodies to determine the seropositivity of all individuals sampled. Surveys were conducted to assess general environmental conditions and demographics for each household. Risk factor and demographic variables were compared using a chi-square and t tests in SAS, accounting for the two-stage cluster sampling methods implemented. Overall LF seroprevalence was 16.01%. Kenema, Port Loko, and Tonkolili districts were 20.12%, 14.14%, and 10.61% seropositive, respectively. We found that individuals were more likely to be LF seropositive if the condition of their toilet is poor ($p < 0.001$), if their household is farther from the water source than other households ($p < 0.001$), if water and food is stored in an individual's room ($p < 0.05$), and if there are rodent holes present in the housing structure ($p < 0.001$). Males were significantly more likely to be seropositive than females ($p < 0.001$) in all three districts ($p < 0.001$). This seroprevalence study provides a general baseline of seropositivity by district. This knowledge will help to inform future epidemiological, ecological, and clinical studies on LF in Sierra Leone.

637

DETECTION OF EBOLA VIRUS FROM OPEN SORES AFTER CLEARANCE OF VIREMIA

Austin D. Ericson[1], Kasereka M. Claude[2], Kambale M. Vicky[2], Tumba Lukaba[3], Michael T. Hawkes[4]

[1]*University of Alberta, Edmonton, AB, Canada,* [2]*Université Catholique du Graben, Butembo, Democratic Republic of the Congo,* [3]*Director of Ebola Virus Disease Case Management, Ministry of Health, Butembo, Democratic Republic of the Congo,* [4]*Department of Pediatrics, University of Alberta, Edmonton, AB, Canada*

The ongoing outbreak of Ebolavirus disease in the Democratic Republic of the Congo is nearing an end, however there is a high risk of resurgence due to persistence of Ebolavirus in immune-privileged sites during convalescence. Transmission is known to occur through semen of male survivors of Ebolavirus disease and there is concern of transmission through other bodily fluids as Ebolavirus has also been identified in breast milk, aqueous humor and urine following clinical recovery and clearance of viremia. Although it has not been previously reported, Ebolavirus may persist in open sores as a result of reduced Ebolavirus-specific immunity at sites of inflammation and sustained infection of macrophages and dendritic cells. Here, we present three cases of Ebolavirus disease with evidence of Ebolavirus RNA in open sores following the clearance of viremia. This finding is significant for infection prevention and control given that open sores are previously unrecognized as a potential source of disease transmission.

638

HIGHLY PATHOGENIC AVIAN INFLUENZA VIRUS SURVEILLANCE IN NORTHERN VIETNAM

Jose Adrian Garcia[1], Bui Nghia Vuong[2], Hung Nguyen-Viet[3], Greg Gray[4]

[1]*NAMRU-2, Phnom Penh, Cambodia,* [2]*National Institute of Veterinary Research, Hanoi, Viet Nam,* [3]*International Livestock Research Institute, Hanoi, Viet Nam,* [4]*Duke University, Durham, NC, United States*

Highly pathogenic avian influenza (AI) virus outbreaks pose a serious threat to both poultry and human health. In early 2017, a 5th wave of Influenza A/H7N9 AI virus infections in humans demonstrated the increasing spread of the virus among poultry in China. Such outbreaks have the ability to cross over and infect neighboring countries via live bird markets (LBMs) and perhaps become enzootic among local aquatic birds and poultry. This was seen in 1997 when a highly pathogenic A/H5N1 outbreak was reported in Hong Kong which soon became widespread among poultry in China and later other SE Asian nations. As a result, there is growing concern that H7N9 will also become enzootic among poultry in Vietnam and cause more frequent infections in humans. Given the limited understanding of AI circulating in Vietnam, active surveillance for influenza A viruses was conducted over a 12-month period between April 2019-March 2020 at Hanoi's largest bird market and in two live bird markets sites near Vietnam's northern border with China. A One Health approach was taken in sample collection which included a total of 433 poultry worker nasal washes, 442 poultry oropharynges swabs, 663 poultry cage swabs and 376 bioaerosol samples. Preliminary data demonstrated that the prevalence of influenza A detected via PCR among sample types was: 4.15% (18/433) in human nasal washes, 37.1% (164/442) in poultry oropharyngeal swabs, 30.1% (200/663) in poultry cage swabs and 40.7% (153/376) in bioaerosol samples. Positive specimens were also cultured in eggs and roughly 15% (182/1176) yielded FluA viruses thus demonstrating the high prevalence of influenza circulating within LBMs. Influenza subtype testing and sequence analysis resulted in a total of 79 recovered sequences representing a very diverse set of H-positive sequences: 4 H3 strains, 1 H4 strain, 2 H5 strains, 15 H6 strains and 57 H9 strains. Further viral characterization is ongoing but current data indicate a wide variety of FluA viruses currently circulating within LBMs in Northern Vietnam.

639

CORRELATION BETWEEN CYCLE-TO-THRESHOLD VALUES FOR INFLUENZA AND CLINICAL SEVERITY IN KAMPHAENG PHET, THAILAND

Kanyarat Phutthasophit[1], Sopon Iamsirithaworn[2], Benjawan Khuntirat[1], Chonticha Klungthong[1], Anthony R. Jones[1], Stefan Fernandez[1], Darunee Buddhari[1]

[1]*USAMD-AFRIMS, Bangkok, Thailand,* [2]*Ministry of Public Health, Nonthaburi, Thailand*

The Department of Virology, USAMD-AFRIMS has conducted influenza surveillance in Kamphaeng Phet since 2012. Nasal and throat swab were collected to identify Flu A (pdmH1 and H3) and Flu B influenza subtypes. Respiratory samples positive for influenza virus were measured by cycle-to-threshold value (Ct <38). This presentation aims to analyze the correlation between Ct values and clinical severity as classified by upper (URI) and lower (LRI) respiratory infection by either chest x-rays finding or clinical diagnosis. We included 905 participants who had positive influenza tests via RT-PCR from April 2012 to December 2019. The median age was 8.6 years (range from 6 months-72 years), 53.5% were male, and 89.1% were diagnosed with an upper respiratory tract infection. RT-PCR revealed that of the samples analyzed 36.7% were Flu B, 32.2% were Flu A/H3, 30.9% were Flu A/pdmH1, and 0.2% were Flu A and Flu B co-infections. The

data were analyzed by descriptive statistics and compared between groups by logistic regression analysis. The baseline age and gender did not differ between groups (p = 0.842 and 0.272). The overall Ct values of upper and lower respiratory diagnosis were significantly different (p< 0.001) at 20.5 (95%CI 20.1, 20.9) and 23.7 (95%CI 22.6, 24.7), respectively. Specifically, the URI and LRI Ct values of Flu B were 18.4 (95%CI 17.9, 18.9) and 21.6 (95%CI 19.9, 23.2), Flu A/H3 were 19.2 (95%CI 18.6, 19.7) and 22.3 (95%CI 20.4, 24.2), and Flu A/pdm H1 were 24.4 (95%CI 23.9, 25.0) and 26.5 (95%CI 25.0, 28.0) respectively. There were significant differences between the URI and LRI for each subtype at p < 0.001. The Ct values of URIs were significantly lower than that of LRIs in all subtypes which suggests that nasal and throat swabs may be more appropriate to measure upper respiratory tract viral infection than the lower ones. These findings could potentially be utilized in formulating collection strategies for emerging disease such as SARS-CoV-2 in the near future.

642

DETERMINATION OF RISK FACTORS AND SPATIAL DISTRIBUTION OF RABIES-POSITIVE DOGS IN CAMBODIA USING CONVENTIONAL AND BAYESIAN REGRESSION MODELLING

Jerome Nicholas Baron[1], Veronique Chevalier[2], Sowath Ly[3], Didier Fontenille[3], Philippe Dussart[3], Malen Chan[3], Beatriz Martinez-Lopez[1]

[1]*University of California Davis, Davis, CA, United States,* [2]*CIRAD, Universite de Montpelier, Montpelier, France,* [3]*Institut Pasteur du Cambodge, Phnom Penh, Cambodia*

With around 800 human deaths per year, Cambodia is one of the countries most affected by rabies worldwide. These estimates rely on decision tree analyses based on passive surveillance at the Institut Pasteur du Cambodge (IPC) in Phnom Penh. These reports are affected by the centralized nature of the surveillance system, with most reported cases and bite injuries being from Phnom Penh and its surroundings. As no direct records of human rabies cases are currently being collected, surveillance relies on voluntary reporting of dog bite injuries and suspect dogs, which are tested. During 2000 to 2016, some 290,000 patients came to IPC seeking post-exposure prophylaxis (PEP) following a dog bite or other animal related injury. For each patient, characteristics of the attack and animal were collected, and for 4,500 cases, an animal was brought to IPC for testing, of which approximately 60% were positive. This study seeks to estimate the likely risk of rabies exposure in IPC patients based on described characteristics, and together with another study assessing the unmet need for PEP, establish a human burden of disease in Cambodia. The data include the individual record associated with each tested animal. Using logistic regression modeling, we assessed attack characteristics associated with positive animals taking into account spatio-temporal distribution of observations. We compared different modelling approaches to choose the best-fitting model. Preliminary results showed that the test status of a dog was strongly associated with the reported health appearance of the animal, but also with other characteristics, such as known ownership of the animal, species, location and severity of injury, number of victims and spontaneity of the attack. Models were then used to predict risk of exposure in IPC patients without tested dogs and estimate the proportion of at PEP patients actually exposed to a rabid animal in each province of Cambodia. This study is part of a broader effort to model rabies and interventions in Cambodia, helping to determine resource allocation, risk-based strategies, and guide policies to meet eradication targets.

643

BEYOND HYDROXYCHLOROQUINE: DISSECTING SARS-COV-2 FUNCTIONAL DRUGGABILITY THROUGH MULTI-TARGET CADD SCREENING OF REPURPOSABLE DRUGS

Yash Gupta[1], Dawid Maciorowski[2], Raman Mathur[1], Ajay Bharti[3], Daniel Becker[2], Brijesh Rathi[4], Ravi Durvasula[1], Steven Bradfute[5], **Prakasha Kempaiah**[1]

[1]*Loyola University Chicago Stritch school of Medicine, Maywood, IL, United States,* [2]*Loyola University Chicago, Chicago, IL, United States,* [3]*University of California, San Diego, NM, United States,* [4]*Loyola University Chicago Stritch school of Medicine and Hansraj College University Enclave, University of Delhi, Maywood, IL, United States,* [5]*University of New Mexico, Albuquerque, NM, United States*

The emergence of SARS-CoV-2 has recently been declared a deadly pandemic causing economic chaos and significant health problems. Like all coronaviruses, SARS-CoV-2 is a large virus has many druggable components. In this study, we have focused on repurposing approved drugs by identifying potential drugs to effectively inhibit SARS-CoV-2. We shortlisted seven target proteins with enzymatic activities known to be essential at different stages of the viral life cycle. For virtual screening, the energy minimization of a crystal structure of the modeled protein was carried out using the Protein Preparation Wizard (Schrödinger LLC, 2020-1). Following active site selection based on data mining and COACH predictions, we performed a HT virtual screen of drugs (n=5900) that are already approved by worldwide regulatory bodies including FDA. The screening was performed against viral targets using three sequential docking modes (i.e. HTVS, SP, and XP). Preliminary in-silico virtual screening identified ~290 potential drugs. Drugs specific to each target protein were further analyzed for binding free energy perturbation by molecular mechanics and pruning the hits to the top 32 candidates. A top leader from each target group was further subjected to molecular dynamics simulation. All the simulated hit-target complexes were found to be strongly interacting and exhibiting highly stable binding. We further tested 20 top hits using a SARS-CoV-2 rescue assay with a Vero cell line treated at 10μM drugs and measured by cell viability assay. Briefly, drug compounds were added 1 h before infection of Vero E6 cells with SARS-CoV-2 and incubated for 48 hours, at which point MTT was added and O.D. measured to calculate the %age of viable cells remaining from viral lysis. Through these experiments, we found potent inhibitors name (%age rescue): Bisindolylmaleimide (42.6%), Haloperidol (30.55%), Doxifluridine(22.25%), Vidarabine(21.06), Hesperidin (21.4%) and Danoprevir(19.25%). Follow-up studies will continue to identify inhibitors suitable for combination therapy based on drug-drug synergy to thwart resistance.

644

VARIATIONS IN MALARIA PREVALENCE AND DRUG RESISTANCE PATTERNS PRE- AND POST- EBOLA VIRUS DISEASE OUTBREAK

Rania AbdelKhalek Nada[1], Isabelle Nakhla[1], Fatorma Bolay[2], Joseph Kowo[3], Josiah George[3], Hanan El Mohammady[1]

[1]*US. Naval Medical Research Unit-3, Cairo, Egypt,* [2]*Liberian Institute for Biomedical Research, Monrovia, Liberia,* [3]*Armed Forces of Liberia, Monrovia, Liberia*

When the Ebola virus disease outbreak (EVD) devastatingly impacted Liberia with thousands of cases and deaths, the World Health Organization recommended empirical use of artemisinin-combined therapy (ACTs) for all fever patients without malaria testing, which may have applied selective pressure for development of ACT resistance in *Plasmodium falciparum*. We aimed to study variations in malaria prevalence and drug-resistance in Armed Forces of Liberia personnel at Edward Binyan Kessely military barracks pre and post EVD. In 2014, pre EVD, 518 dried blood spots (DBS) were collected from asymptomatic subjects enrolled in a serosurvey. In 2015, after EVD, another set of DBS from 557 subjects was collected. Testing with qPCR revealed a *Plasmodium* genus in 37(7.1%)

of pre EVD samples, of which 31(83.7%) were *P. falciparum*, 4(10.8%) *P. ovale* and two (5.4%) *P. malariae*. In post EVD samples, *Plasmodium* genus was found in 116 (20.8%) samples, in which *P. falciparum* was identified in 62 (53.4%) only. An additional 122 malaria patients were enrolled to better study pre and post EVD changes in *dhfr* and *dhps* genes for sulfadoxine/pyrimethamine (SP) resistance and k13 gene for ACT resistance. Conventional PCR amplified products in 25 pre- and 26 post EVD samples, and 91 malaria patients. Pre EVD: 84% of strains showed ≥4 dhfr+dhps mutations; a wild-type k13 gene was shown in all except one mutant strain (V581I). Post EVD: 98.2% of strains showed ≥4 dhfr+dhps mutations. A set of novel mutations was identified: Two new *dhfr* mutations, R38S (n=2) and V65R (n=1); five new *dhps* mutations, H614L (n=7), I484T, I441M and I431V each in two strains, and I429L in one strain. For k13 gene, three strains showed k13 gene alleles, two of which had synonymous mutations (C469C & Q613Q) and one had one non-synonymous mutation (V555A). Our data shows an increase in *Plasmodium* genus and mutations conferring resistance against SP, as well as, emerging new mutations in genes conferring malaria drug resistance. Follow up studies are essential to monitor epidemiologic and genetic changes in *Plasmodium* strains in Liberia.

645

TEMPORAL TRENDS OF *PLASMODIUM FALCIPARUM* MULTI-DRUG RESISTANCE PROTEIN 1 GENE DURING IMPLEMENTATION OF ARTEMISININ COMBINATION THERAPIES BETWEEN 2008 AND 2019 IN KENYA

Winnie Adhiambo Okore[1], Raphael Okoth[1], Edwin Mwakio[1], Redemptah Yeda[1], Benjamin Opot[1], Jackline Juma[1], Gladys Chemwor[1], Charles Okello[1], Agnes Cheruiyot[1], Dennis Juma[1], Hoseah Akala[1], Collins Ouma[2], Ben Andagalu[1], Amanda Roth[1]

[1]*US Army Medical Research Directorate-Africa/KEMRI, Kisumu, Kenya,* [2]*Maseno University, Kisumu, Kenya*

Single nucleotide polymorphisms (SNPs) in the *Plasmodium falciparum* multi-drug resistance protein 1 (Pfmrp1) gene have previously been associated with conferring resistance against artemisinin and its partner drugs in Southeast Asia. With no suitable replacement for artemisinin combination therapies (ACTs), establishing frequency of these polymorphisms contributing to impaired response to ACTs treatment is key for continued drug resistance surveillance in Africa, where the putative Kelch propeller polymorphisms are not yet identified. 300 samples collected from 6 sites between 2008 and 2019, under an ongoing epidemiology of malaria and drug resistance sensitivity patterns in Kenya study. Were assayed for SNPs at Pfmrp1 gene codons; H191Y, S437A, I876V and F1390I using Agena MassARRAY platform. Field isolates were also tested against artemisinin (ART), lumefantrine (LU), amodiaquine (AQ), mefloquine (MQ), quinine (QN) and quinine (CQ) to determine their *in vitro* drug sensitivity using the malaria SYBR Green I-based fluorescence assay. Reference clones were tested in parallel as the assay standards. Categorical data was analyzed as proportions showing rates of frequency and median IC_{50} values. Of the 139 samples typed, polymorphisms at Pfmrp1 codons I876V and F1390I were the most frequent at 37% and 1.4% mutants, 17% and 4% mixed, 42% and 89% wild type respectively. The frequency of the I876V polymorphisms increased over time across all the sites. During the same period, LU and AQ had median IC_{50}s values that changed from 14.93 ng/ml [IQR=8.384-19.84, n=29], 15.12 ng/ml [IQR=5.352-38.74 n=55], 27.33 ng/ml [IQR=4.078-46.91 n=45] P<0.03, 2.981 ng/ml [IQR=2.566-4.764 n=50] 1.607 ng/ml [0.9586-2.037 n=43], 1.101 ng/ml [IQR 0.5348-3.052 n=47] P<0.0001 respectively. Across time LU showed increase in IC50s and AQ showed decrease in the IC50s. Study findings showed an association between Pfmrp1 SNP codon I876V and *in vitro* response to LU, MQ and ART with P value <0.0001. Further studies are needed to better understand the trend of antimalarial resistance associated with Pfmrp1 gene in Africa.

646

A TREND OF IN-VITRO ANTIMALARIAL PERFORMANCE IN A SPAN OF TEN YEARS DURING ARTEMISININ COMBINATION THERAPY IN KENYA

Agnes Cheruiyot, Hosea Akala, Redemptah Yeda, Charles Okudo, Dennis Juma, Benjamin Opot, Edwin Mwakio, Jackline Juma, Raphael Okoth, Gladys Chemwor, Ben Andagalu, Amanda Roth

USAMRU-K, Kisumu, Kenya

Emergence of antimalarial drug resistance in Southeast Asia is a major obstacle in elimination of malaria, thus need for continued surveillance. *In vitro* testing of *Plasmodium* isolates provides an additional platform to monitor susceptibility to antimalarials that influences malaria drug prescription. Susceptibility data from an ongoing surveillance study of malaria and drug sensitivity patterns in Kenya were further analyzed from 2008 to 2019. Antimalarials screened included: chloroquine (CQ), quinine (QN), atovaquone (AV), primaquine (PQ) and mefloquine (MQ). Inhibition curves in, *in vitro* assays, were obtained from the relative fluorescence units (RFU) using Graph Pad Prism. Trend in CQ median IC_{50} was 17.33ng/ml (95% CI, 2.863 to 42.54) in 2008, 6.903 ng/ml (95% CI, 0.4013 to 62.06) in 2013 and decreased to 5.646 ng/ml (95% CI, 1.272 to 19.25), p<0.0001 in 2019. AV median IC_{50} was 1.280ng/ml (95% CI, 0.2242 to 4.072) in 2008, 0.6612 ng/ml (95% CI, 0.1390 to 7.210) in 2013 and decreased to 0.600 ng/ml 8(95% CI, 0.0610 to 3.534), p<0.0001 in 2019. QN median IC_{50} was 68.05ng/ml (95% CI, 14.16 to 362.4) in 2008, 61.84ng/ml (95% CI, 1.423 to 1306) in 2013 and 13.48 ng/ml (95% CI, 0.4279 to 152.6), p<0.0001 in 2019. PQ median IC_{50} was 367.5ng/ml (95% CI, 54.45 to 973.3) in 2009, 333.8ng/ml (95% CI, 4.842 to 1010) in 2013 and 36.36 ng/ml (95% CI, 1.650 to 1137), p<0.0001 in 2019. MQ median IC_{50} was 5.208ng/ml (95% CI, 0.5840 to 13.57) in 2008, 3.187 ng/ml (95% CI, 0.1680 to 12.36) in 2013 and 4.855 ng/ml (95% CI, 0.8768 to 10.37), p=0.0030 in 2019. Results show a reducing median in antimalarials tested suggesting higher susceptibility to *P. falciparum* strains. A lower median (ng/mL) observed over years suggests improved susceptibility of antimalarials hence this data may support their continued usage, for the drugs that have been approved by the ministry of health. This study appears to underscore the importance of surveillance studies using sustainable methods to provide data on *Plasmodium* isolate susceptibility to antimalarials.

648

RETENTION OF NON-FALCIPARUM SPECIES AFTER ACT TREATMENT COULD BE RESPONSIBLE FOR RISING CASES OF EXPORTED MALARIA GLOBALLY

Gladys C. Chemwor, Hoseah M. Akala, Benjamin Opot, Raphael Okoth, Redemptah Yedah, Jackline Juma, Agnes Cheruiyot, Dennis Juma, Ben Andagalu, Amanda Roth

Department of Emerging Infectious Diseases (DEID), United States Army Medical Research Directorate-Africa Kenya (USAMRD- K), Kenya Medical Research Institute (KEMRI), Kisumu, Kenya

Mixed malaria species infections are increasing. However, vague data on the response of non-*falciparum* species to the Artemisinin Combination Therapy (ACT) in relation to transmission is available. We compared the response of mixed versus single *Plasmodium* species infections to ACT. 528 blood samples were collected on days 0, 7, 14, 21, 28, 35, and day 42 from 88 individuals enrolled in an ACT efficacy study between 2013 and 2015 in Kisumu, Western Kenya. All positive samples were typed for *Plasmodium* species composition using species-specific primers for *Plasmodium falciparum* (*Pf*), *P. malariae* (*Pm*), *P. ovale curtisi* (*Poc*) and *P. ovale wallikeri* (*Pow*) in a 18s rRNA based real-time PCR. Recurrent parasitemia were characterized for species composition and treatment outcome was monitored during 42-day follow-up period. 85% (70/82) of the day 0 samples were *Pf* single species infections while 14% (12/82) were multiple species infections containing *Pf*/*Pm*, *Pf*/*Pow* & *Pf*/*Poc*/*Pm* at 6% (5/82), 4% (4/82) & 3% (3/82) respectively. Day 28 treatment

outcomes showed that *P. falciparum* single species infections had a higher prevalence of Adequate Clinical and Parasitological Response (ACPR) at 75% (53/70) than mixed-species at 58% (7/12). Additionally, mixed-species infections had a higher percentage of Late Clinical Failure (LCF) at 33% (4/12) than single *Pf* at 20% (14/70) similar to outcome 42 at 41% (5/12) for the mixed species and 40% (28/70) for mono *falciparum*. The median parasite clearance slope half-life for single *Pf* species versus mixed-infections were 2.32 (0.97 - 3.60) hours and 2.77 (1.78 - 4.21) hours respectively. The median time taken to clear 99% of the parasite load for the single *P. falciparum* infection and mixed-infections were 18.57 and 22.26 hours respectively. Findings show slow response of non-*pf* to ACTs depicted by discernible increase in frequency of these species during the 42-day follow-up period. The observed retention of non-*pf* species especially *Pow* for the entire treatment period appears to argue for notable rise of cases of exported malaria globally.

649

THE *PFCORONIN* GENE IS HYPOSTATIC TO *PFKELCH13*

Aabha I. Sharma, **Sara H. Shin**, Selina Bopp, Sarah K. Volkman, Daniel L. Hartl, Dyann F. Wirth

Harvard T. H. Chan School of Public Health, Boston, MA, United States

We sought to investigate the genetic determinants of Artemisinin (ART) resistance in the African context, where the highest burden of malaria exists. Previously, we used Pikine and Thiès Senegalese isolates to perform *in vitro* evolution and generated two independent ART-resistant *Plasmodium falciparum* parasite lines. Using whole genome sequencing, we identified mutations in the actin-bundling protein, *Pf*Coronin in both the evolved parasite lines. To evaluate the gain and loss of function in resistance associated with mutations in *pfcoronin*, we generated CRISPR-Cas9 edited parasites and established *Pf*Coronin to be the major driver of ART resistance *in vitro.* Considering the role of actin in endocytosis, the pathway recently found to be disrupted in *Pf*Kelch13-mediated ART resistance, we studied the potential resistance synergy between mutations of *Pf*Coronin and *Pf*Kelch13. We generated single and double mutant parasites with *pfcoronin* (R100K & E107V) and *pfkelch13* (C580Y) mutations in the Pikine and 3D7 genetic backgrounds. This study is the first report of *pfkelch13* mutants in the Pikine background, which had significantly higher survival in the Ring Stage Survival Assay (RSA) in two clones (28% and 42%) relative to the wildtype (RSA<1%). C580Y mutants in the Pikine background were also fitness neutral, suggesting that they could be competitive in the field. RSA survival observed in two clones of the *pfcoronin* and *pfkelch13* double mutant parasites (27% and 33%) were similar to those of *pfkelch13* mutants in the Pikine background, suggesting the *pfcoronin* gene to be hypostatic to *pfkelch13.* This phenomenon was also observed in the 3D7 background, implying that *pfcoronin* could mediate resistance downstream of *pfkelch13* in the same pathway. Given that *Toxoplasma gondii* Coronin is involved in vesicular trafficking independent of its function in actin binding, *Pf*Coronin could disrupt endocytic machinery without actin bundling to confer ART resistance. Future studies on *Pf*Coronin biology as well as epistatic interactions between the other players of resistance could elucidate unique pathways exploited by the parasites to become resistant.

650

MODELING TRANSPORT OF ANTIMALARIALS AND PEPTIDES BY PIPERAQUINE-RESISTANT MUTANT PFCRT

Laura M. Hagenah, Jonathan Kim, Eva G. Iturbe, Matthias Quick, Filippo Mancia, David A. Fidock

Columbia University Irving Medical Center, New York, NY, United States

International efforts to control and eventually eliminate *Plasmodium falciparum* malaria have been thwarted by the emergence of resistance to first line antimalarials. In Southeast Asia, piperaquine (PPQ), used as a combination therapy with dihydroartemisinin (DHA), has encountered widespread resistance, mediated by single amino acid substitutions in the Dd2 isoforms of the *Plasmodium falciparum* Chloroquine Resistance Transporter (PfCRT). These mutant PfCRT isoforms have evolved to enable efflux of PPQ away from its site of action in the parasite's digestive vacuole, allowing for survival even at high PPQ concentrations. Although these mutations are on the chloroquine (CQ) resistant Dd2 background, most re-sensitize parasites to CQ. It is not understood why these specific single amino acid changes result in this phenomenon, as well as how these mutations impact the native function of the transporter. Here we use *in vitro* binding and transport studies with purified protein to characterize the mechanism of transport mediated by PPQ-resistant (PPQ-R) mutant PfCRT. Various contemporary, globally-relevant PPQ-R PfCRT isoforms were expressed in HEK293 cells using baculovirus gene transfer. Purified protein and tritiated drug were used to assess the binding affinity of PPQ for these PfCRT isoforms, using scintillation-proximity based binding assays. ^{3}H-PPQ uptake assays with PPQ-R PfCRT-containing proteoliposomes showed PPQ transport in a membrane potential- and pH-dependent manner, consistent with an active efflux mechanism that drives resistance to PPQ. These PfCRT isoforms have reduced CQ transport compared to Dd2 PfCRT, suggesting that distinct mechanistic features mediate the resistance to CQ and PPQ in PfCRT. Ongoing studies will reveal the effect of these PPQ-R mutations on the levels of globin-derived peptide transport via PfCRT. This study provides important insights into the molecular basis of antimalarial drug resistance, of direct relevance to the goal of reducing the morbidity, mortality, and socioeconomic burden of malaria.

651

COLLATERAL SENSITIVITY AS A STRATEGY TO SUPPRESS RESISTANCE: THE CHALLENGE OF DIVERSE EVOLUTIONARY PATHWAYS

Rebecca E. Mandt[1], Maria Jose Lafuente-Monasterio[2], Madeline R. Luth[3], Sabine Ottilie[3], Elizabeth A. Winzeler[3], Francisco Javier Gamo[2], Amanda K. Lukens[1], Dyann F. Wirth[1]

[1]*Harvard TH Chan School of Public Health, Boston, MA, United States,* [2]*Tres Cantos Medicines Development Campus, GlaxoSmithKline, Madrid, Spain,* [3]*University of California San Diego, La Jolla, CA, United States*

Collateral sensitivity—when resistance to one drug causes increased sensitivity to another chemical agent—has been explored as a strategy to suppress resistance in bacteria, viruses, parasites and even cancer. Previously, we characterized development of resistance to the antimalarial candidate DSM265, a dihydroorotate dehydrogenase (DHODH) inhibitor, using *in vitro* cell culture as well as a mouse model of *Plasmodium falciparum* infection. We identified several point mutations in *dhodh* that confer resistance, including DHODH C276Y, which also arose in clinical trials. Here, we report parasites resistant to DSM265 exhibit collateral sensitivity to other structurally distinct DHODH inhibitors. We treated parasites with combinations of DSM265 and the DHODH inhibitor Genz669178. Because some mutations, including DHODH C276Y, confer cross-resistance to both chemotypes, we found that resistance arose readily *in vitro.* Interestingly, resistance to this combination did not develop in the mouse model, suggesting *in vivo* pharmacological parameters can influence resistance evolution. We also screened a broader set of structurally diverse DHODH inhibitors, and found one compound, TCMDC-125334, active against all mutant lines tested. We hypothesized treating parasites with DSM265 and TCMDC-125334 in combination would suppress the emergence of *in vitro* resistance. However, as previously presented, while resistance was delayed, cross-resistance to both compounds eventually emerged, conferred by the DHODH V532A mutation. We find that DHODH V532A parasites are relatively fit in *in vitro* competition assays, suggesting they are evolutionarily viable. We also wanted to test whether treating DHODH C276Y parasites, which are ~10-fold hypersensitive to TCMDC-125334, with this compound, would cause a reversion to wildtype sequence. Surprisingly, TCMDC-1253334- selected C276Y parasites gained additional genetic changes in *dhodh* making them less sensitive to TCMDC-125334 and conferring high level (>100-fold) resistance to DSM265. Overall, we highlight the mutational flexibility of DHODH and its liability as a malaria drug target.

653

EFFICACY AND SAFETY OF ARTESUNATE-AMODIAQUINE FOR THE TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA IN MAINLAND TANZANIA

Twilumba Makene[1], Billy Ngasala[1], Samwel Bushukatare[1], Mercy Chiduo[2], Erasmus Kamugisha[3], Maimuna Ahmed[3], Muhidin Mahende[4], Reginald Kavishe[5], Florida Muro[5], Debora Kajeguka[5], Deus Ishengoma[2], Celine Mandara[2], Ritha Njau[6], Ssanyu Nyinondi[7], Bilal Kabula[7], Frank Chaky[8], Ally Mohamed[8]

[1]*Muhimbili University of Health and Allied Sciences, Dar es Salaam, United Republic of Tanzania,* [2]*National Institute for Medical Research, Tanga, United Republic of Tanzania,* [3]*Catholic University of Health and Allied Sciences/Bugando Medical Centre, Mwanza, United Republic of Tanzania,* [4]*Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania,* [5]*Kilimanjaro Christian Medical University College, Kilimanjaro, United Republic of Tanzania,* [6]*World Health Organization Country Office, Dar es Salaam, United Republic of Tanzania,* [7]*RTI International,, Dar es Salaam, United Republic of Tanzania,* [8]*National Malaria Control Program, Dodoma, United Republic of Tanzania*

Tanzania NMCP in partnership with WHO recommended the use of ACTs since 2006, among which ALU was adopted as first line anti-malaria treatment. But with the current threat of ALU resistance, regular monitoring of efficacy and safety for alternative ACTs is vital in malaria endemic countries. Surveillance of Artesunate-Amodiaquine-ASAQ (as the alternative ACT in Tanzania) is of paramount importance in this era of emerging ALU resistance and the increased malaria global trend for health decision makers and treatment guidelines. The clinical efficacy of ASAQ in uncomplicated falciparum malaria in mainland Tanzania was assessed in three NMCP sentinel sites over 28 days. A one arm study was conducted in Mlimba, Nagaga and Mkuzi sites, and out of 628 screened, only 264 (39.4%) met the inclusion criteria for the study as per WHO protocol recommendations and were microscopically confirmed with uncomplicated Malaria. 1(0.4%) was lost to follow up and 1(0.4%) withdrew from the study. Adequate clinical and parasitological response was primary endpoint measure for the study with 262 (99.9%) patients completing 28 days follow up. Parasitemia was cleared within 3 days of medication administration in all participants, and only 1(0.4%) early treatment failure was observed, late clinical failure occurred in 1 (0.4%) patient, and late parasitological failure occurred in (0.4%) patients. ASAQ had a parasite cure rate of 98.9%. PCR-uncorrected ACPR on day 28 was high for more than 96.6% in all sites (96.6% at Mkuzi, 98.6 % at Nagaga and 98.9% at Mlimba. PCR corrected results will be available during annual meeting presentation. There was no serious adverse effect reported. The most common adverse effect was cough (57.1%), and it was not associated with the antimalarial drug. In conclusion these findings confirm high efficacy and safety of the recommended ASAQ (alternative ACT) for treatments for uncomplicated falciparum malaria.

654

ARTESUNATE-AMODIAQUINE AND ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA IN LIBERIAN CHILDREN: IN VIVO AND POLYMORPHISMS OF *PFK-13 2017-2018*

Victor S. Koko[1], Marian Warsame[2], Benjamin Vohm[3], Moses Jeuronlon[4], Fahn Taweh[3], Lekilay Tehmeh[5], Paye Nyansaiye[1], Oliver Pratt[1]

[1]*Ministry of Health/National Malaria Control Program, Monrovia, Liberia,* [2]*Gothenburg University, Sweden, Sweden,* [3]*National Public Health Institute of Liberia-NPHIL, Monrovia, Liberia,* [4]*World Health Organization (WHO), Monrovia, Liberia,* [5]*Quality Control Unit/Ministry of Health, Monrovia, Liberia*

Malaria is a major public health problem in Liberia accounting for 42% and 39% OPD and IPD attendance.Artesunate-amodiaquine (ASAQ) and artemether-lumefantrine (AL) are the first-line treatments for uncomplicated falciparum malaria in Liberia. WHO recommends regular monitoring of efficacy of nationally recommended artemisinin-based combinations (ACTs).This study aimed to assess the efficacy of these ACTs and markers of artemisinin resistance.A single arm prospective study was conducted to assess the efficacy of ASAQ (Bensonville-South Central region and Saclepea-North Central region sites) and AL (Sinje-Western region and Kakata sites-South Central region) among 6-59 months old children with uncomplicated falciparum malaria. Mutations in k13 gene associated with artemisinin resistance were investigated. The study outcomes were PCR corrected adequate clinical and parasitological response, day 3 positivity rate and molecular markers of artemisinin resistance. Out of the 359 enrolled children, 180 were treated with ASAQ (89 in Saclepea and 91 in Bensonville) and 179 with AL (90 in Sinje and 89 in Kakata).Of the recruited children, 332 provided study endpoints. Among the ASAQ treated group, PCR corrected ACPR of 91.8% and 92.7% in Sacleapea and Bensonville, respectively as per-protocol analysis. For the AL treated group, 100% PCR corrected ACPR was observed in both sites. All patients, but two in Sinje, cleared their parasitaemia on day 3. A total of 356 D0 samples were tested *Pfk13* mutation and 349 gave interpretable data (98%). The analysis did not show non-synonymous mutation. All patients treated with AL achieved adequate clinical and parasitological response (ACPR). With ASAQ 91.8% of the patients achieved ACPR, just above the 90% threshold under which change of treatment is recommended. This calls for close monitoring of ASAQ. No validated *PfK13* mutant was observed.

655

SAFETY AND EFFICACY OF PYRONARIDINE-ARTESUNATE AND NEW DRUG COMBINATIONS WITH ATOVAQUONE-PROGUANIL FOR THE TREATMENT OF DRUG RESISTANT *P. FALCIPARUM* MALARIA IN CAMBODIA

Mariusz Wojnarski[1], Chanthap Lon[1], Somethy Sok[2], Darapiseth Sea[1], Threechada Boonchan[1], Michele Spring[1], Nillawan Buathong[1], Soklyda Chann[3], Pattaraporn Vanachayangkul[1], Nonlawat Boonyalai[1], Chandara Sok[4], Sabaithip Sriwichai[1], Nichapat Uthaimonkol[1], Kimberly A. Edgel[5], Tyler Warkentien[5], Catherine Berjohn[5], Paphavee Lertsethtakarn[1], Piyaporn Saingam[1], Chaiyaporn Chaisatit[1], Kittijarankon Phontham[1], Worachet Kuntawunginn[1], Dennis Faix[5], Prom Satharath[2], Jurgen Venitz[6], Pascal Ringwald[7], Rekol Huy[8], Krisada Jongsakul[1], Philip Smith[1], John S. Brooks[5], Nicholas J. Martin[1], Mark M. Fukuda[1], Dysoley Lek[8], Norman Waters[1]

[1]*Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand,* [2]*Ministry of National Defense, Department of Health, Phnom Penh, Cambodia,* [3]*Chenda Polyclinic, Phnom Penh, Cambodia,* [4]*Armed Forces Research Institute of Medical Sciences, Phnom Penh, Cambodia,* [5]*Naval Medical Research Unit 2, Phnom Penh, Cambodia,* [6]*Virginia Commonwealth University, Richmond, VA, United States,* [7]*World Health Organization, Geneva, Switzerland,* [8]*National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia*

Multi-drug resistant *Plasmodium falciparum* (*P.f*) malaria in Southeast Asia underscores the need for more effective drug combinations in areas approaching malaria elimination, where malaria may become untreatable by existing first-line treatments. The main objective of the study was to assess the therapeutic efficacy and tolerability of artesunate-pyronaridine (ASPY) and novel drug combinations of ASPY+atovaquone-proguanil (ASPY+AP) and artesunate-mefloquine+atovaquone-proguanil (ASMQ+AP) in patients with uncomplicated *P.f*. The study is being conducted at 3 sites in Cambodia, under directly observed therapy and a minimum of 3 days of hospitalization, with 8 weeks of follow up. Volunteers assigned to ASPY and ASPY+AP take the study drugs over 3 day period, while volunteers assigned to ASMQ+AP take ASMQ during the first 3 days, followed by AP on days 3-6. To date, of a planned enrollment target of 252, 78 volunteers have been enrolled and completed 8 weeks of follow up. All volunteers had adequate clinical parasitological response for their primary treatment outcome. Transient elevations of transaminase levels were reported for volunteers treated with ASPY and ASPY+AP but all had documented rapid resolution of the elevated ALT on follow up. None of the volunteers

met the Hy's law criteria. Mild increases in QTc values were observed for the combinations, consistent with prior reports for antimalarials. The volunteers assigned to sequential treatment of ASMQ followed by AP had the greatest number of neurological and GI side effects, attributed to mefloquine. In conclusion, based on the preliminary data, the studied drug combinations appear safe in most volunteers, with high cure rate. Addition of AP to artemisinin-based combination treatments did not increase the risk of hepatotoxicity, cardiac liability or GI intolerance but mefloquine was associated with higher rate of side effects. Information on drug resistance as well as additional safety, efficacy, and tolerability data for the studied drug combinations and their corresponding pharmacokinetic-pharmacodynamics interactions will be presented.

656

PRIMAQUINE METABOLISM: PQ-5,6-O-QUINONE AND 6-METHOXYQUINOLINE-5,8-*P*-QUINONE GENERATED IN HUMAN ERYTHROCYTES

NP Dhammika Nanayakkara[1], Washim Khan[1], Yanhong Wang[1], HMT Bandera Herath[1], Islam Hussein[1], Shabana I. Khan[1], Narayan D. Chaurasiya[2], Babu L. Tekwani[2], **Larry A. Walker**[1]

[1]*National Center for Natural Products Research, University of Mississippi, University, MS, United States,* [2]*Southern Research, Birmingham, AL, United States*

Primaquine (PQ), in spite of its unique utility in the treatment of malaria and transmission blockade, elicits hemolytic toxicity in individuals deficient in glucose 6-phosphate dehydrogenase (G6PD). This has severely limited its use in the public health arena. Understanding the mechanisms involved in the hemolytic insult would greatly contribute to safe use of this class. Oxidative metabolites of PQ generated through CYP-mediated pathways have been implicated in efficacy and toxicity of PQ. Previous studies in our labs have demonstrated oxidation of PQ within human erythrocytes to form primaquine-5,6-*ortho*quinone (PQ*o*Q). PQ is apparently oxidized non-enzymatically to 5-hydroxy-PQ (5OHPQ), which further oxidizes spontaneously to PQ-quinone-imine (PQ-QI), or undergoes oxidative demethylation to yield the PQoQ. On our explorations of PQ effects on erythrocytes, LC/MS metabolomics analysis consistently showed a metabolite with mass *m/z* 190, which did not match with *m/z* 190 metabolites suggested in the Human Metabolome Database (HMDB). Employment of stable isotope method, using a mixture of ^{12}C and ^{13}C-labeled PQ (at 6 quinoline core carbons) permitted the confirmation of a new PQ metabolite. Derivation from PQ was confirmed by the twin peaks at *m/z* 190 and 196 (the latter reflecting the ^{13}C label). The product was identified as 6-methoxyquinoline 5,8-*p*-quinone (MQ*p*Q). The compound was synthesized and structure verified by NMR. The metabolite's UPLC retention time and MS/MS fragmentation profile (major fragments of *m/z* 190 at *m/z* 161, *m/z* 147, *m/z* 119) were consistent with the synthetic reference compound. The MQ*p*Q metabolite is likely formed from PQ-QI, via hydrolysis of the imine and loss of the alkylamine side chain. Thus, both PQoQ and MQ*p*Q derive from the central 5OHPQ metabolite formation. Importantly, MQ*p*Q readily formed a covalent adduct when incubated with reduced glutathione, suggesting that it may be a reactive metabolite. In contrast, PQoQ did not form the glutathione adduct under similar conditions. This study further illuminates potential reactive species in the mechanism of hemolytic toxicity of PQ.

657

FREQUENCY OF *PLASMODIUM FALCIPARUM* AND *SCHISTOSOMA MANSONI* CO-INFECTION IN YORO-VILLAGE, CAMEROON: IMPLICATION ON RAPID DIAGNOSIS OF MALARIA

Eric Berenger Tchoupe Kamoua

University of Yaounde 1, Younde, Cameroon

Malaria and schistosomiasis are major parasitic diseases causing morbidity and mortality around the world. Cross-reactive antibodies to different components of the two parasites could influence the diagnostic of malaria parasite. This study is designed to assess the impact of *Plasmodium* and *Schistosoma* co-infection on malaria rapid diagnosis. The study was conducted in a total of 319 children. Malaria rapid diagnostic were performed to confirm malaria cases and Kato Katz method was used to confirm schistosomiasis. PCR was used to determine the level of gene deletion within the population. The prevalence of malaria was 275 and 256 with RDT and microscopy respectively. 116 participants were also infected by Schistosomiasis with a co-infection rate of 30.40%. Of the 44 negative samples obtained with RDT, 10.03 % were noted to be false negative. The sensitivities of RDT and PCR were respectively 87.5 % and 86.71 %. 14 (with 3 co-infections) out of the 250 PCR positive samples were found to have undergone deletion suggesting a possible influence of rapid malaria diagnosis. Despite the high prevalence of Schisto-malaria co-infection, no significant association between co-infection and deletion of the Pfhrp2 gene was found. However, *P. falciparum* and *S. mansoni* Co-infection may influence the rapid diagnosis of malaria.

658

GAZELLE: A PORTABLE POINT-OF-CARE DIAGNOSTIC WITH HIGH ACCURACY AND FAST TURNAROUND TIME FOR DETECTING *PLASMODIUM VIVAX* MALARIA

Rebeca Linhares Abreu Netto[1], Victor Irungu Mwangi[1], Yanka Evellyn Alves Rodrigues Salazar[1], Priyaleela Thota[2], Vanderson de Souza Sampaio[1], Anne Rocheleau[2], Wuelton Marcelo Monteiro[1], Tyler Witte[2], Fernando Fonseca de Almeida e Val[1], Marcus Vinicius Guimarães Lacerda[1], **Gisely Cardoso Melo**[1]

[1]*Fundação de Medicina Tropical Dr Heitor Vieira Dourado, Manaus, Brazil,* [2]*Hemex Health, Portland, OR, United States*

Malaria remains a major public health concern with an estimated 3.4 billion people in 92 countries at risk of being infected with malaria and developing disease. *Plasmodium vivax* is the dominant strain of malaria species in most countries outside of sub-Saharan Africa. Although less virulent than *P. falciparum, P. vivax* can still result in severe complications and death. *P. vivax* infections are characterized by lower parasitemia than *P. falciparum* and are often missed by RDTs, which are less reliable at detecting non-falciparum species. Microscopy can detect lower levels of parasitemia but can take up to 1 hour and accuracy depends on the expertise of the microscopists. Given the practical limitations of microscopy, RDTs and PCR, a high unmet need exists for an improved diagnostic for *P. vivax*. Our study evaluated an inexpensive, and fast (~1 minute) malaria detection device (Gazelle™), using Magneto-Optical Detection (MOD) to detect hemozoin in a blood sample. Gazelle™ is a battery operated, rugged device with a digital interface and incorporates gps, wifi and Bluetooth. Hence, the device can be operated with minimal training in poor resource settings with erratic power supply. Test samples were collected from 300 patients who were enrolled at a reference infectious disease hospital in the Western Brazilian Amazon. Each sample was tested with Gazelle™ and RDT, and the results were compared to microscopy and PCR. Overall, 277 patient samples were included in the analysis (16 were excluded due to inconclusive results and seven due to *P. falciparum* and/or mixed infections). Compared with RDTs, Gazelle™ showed better sensitivity (96.2% vs. 84%), the same specificity (100%) and higher accuracy (98.2% vs. 92%) with microscopy as gold standard and was much faster at one-minute turnaround time than either microscopy or RDTs. When compared to PCR, the accuracy of Gazelle was 82.3%, whereas it was 84.1% for microscopy and 76.5% for RDTs. This study demonstrates that the performance of Gazelle was comparable to expert microscopy and was superior to RDTs. Gazelle™ may be a potential solution for settings where there is a need for speed, accuracy and ease of use.

659

DEVELOPMENT OF THE 1ST WHO REFERENCE REAGENT FOR ANTIMALARIA (*PLASMODIUM VIVAX*) HUMAN PLASMA

Charles Olomu[1], Lynne M. Harris[1], **Seda Yerlikaya**[2], Peter Rigsby[1], Eleanor Atkinson[1], Marta Vidal[3], Ruth Aguila[3], Carlota Dobaño[3], Xavier Ding[2], Paul W. Bowyer[1], the Collaborative Study Group[2]

[1]*National Institute for Biological Standards and Control (NIBSC), Blanche Lane, South Mimms, Hertfordshire, United Kingdom,* [2]*FIND, Geneva, Switzerland,* [3]*ISGlobal, Hospital Clínic - Universitat de Barcelona, Barcelona, Spain*

Immunoassay standards are critical tools to ensure standardization, harmonization, and cross-comparison of immunological assays used in diagnostics and vaccine development and epidemiology research. While a Reference Reagent for anti-*P. falciparum* human serum is available, the malaria research field has been lacking an equivalent reagent for *P. vivax*. FIND and NIBSC have recently conducted an international collaborative study to assess the suitability of lyophilized plasma preparations, containing *P. vivax*-specific antibodies, to serve as the first WHO Reference Reagent for anti-malaria (*P. vivax*) human plasma. Human plasma samples, were obtained from adults with a confirmed *P. vivax* malaria diagnosis. These were pooled and assessed, by ELISA, against *P. vivax* circumsporozoite protein (CSP), Duffy-binding protein (DBP), apical membrane antigen-1 (AMA-1), and merozoite surface protein 1-19 (MSP1-19). An international collaborative study involving 15 participant laboratories evaluated whether the anti-malaria (*P. vivax*) human plasma candidate preparation is fit-for-purpose. The ongoing statistical analysis of the data obtained in the collaborative study will demonstrate whether the reference reagent enables a reduction in inter- and intra-laboratory variability in enzyme-linked immunosorbent assays (ELISA). An accelerated thermal degradation study is in progress to assess the suitability of the candidate reagent in terms of its predicted long-term stability. The results will be presented to the WHO Expert Committee on Biological standardization at the 2020 meeting with the request to establish the 1st WHO Reference Reagent for antimalaria (*Plasmodium vivax*) human plasma. The purpose will be to assist in the standardization of immunological assays used in *P. vivax* diagnostics development, facilitating optimization and validation of such assays in vaccine development and allowing cross-comparison of assay results across products and laboratories.

660

UTILITY OF REPORTING PRESUMED MALARIA FOR IMPROVING MALARIA CASE MANAGEMENT IN MALI

Diadier Amadou Diallo[1], Madina Konaté[2], Ignace Traore[1], Ramine Bahrambegi[3], Assitan Coulibaly[2], Jean-Marie NGbichi[4], Idrissa Cissé[2], Jules Mihigo[5], Yazoume Yé[6]

[1]*ICF, Bamako, Mali,* [2]*National Malaria Control Program, Bamako, Mali,* [3]*ICF, Washington, DC, United States,* [4]*ICF, Chapel Hill, NC, United States,* [5]*United States Agency for International Development - President Malaria Initiative, Bamako, Mali,* [6]*ICF, Rockville, MD, United States*

Malaria treatment guidelines require antimalarial treatment only for patients with a positive diagnostic test for malaria. However, in Mali, patients with negative diagnostic tests or no tests were being given antimalarial drugs, affecting the accurate estimation of the commodities needed. At the end of 2018, Mali revised the reporting form and register to allow reporting of "presumed cases" (cases treated for malaria without parasitological confirmation). After a year (January – December 2019) using the new monthly reporting form, the MEASURE Evaluation project, working with the NMCP, analyzed 2019 routine malaria data from the District Health Information Software, version 2 (DHIS2) platform to understand the extent to which health workers adhered to treatment guidelines and factors affecting their adherence. We found that in 2019, 3.2 million malaria cases (presumed and confirmed) were reported by health center personnel and community health workers (CHWs). Overall, presumed malaria cases represented 12% (385,553) of total malaria cases reported. The five northern regions—Menaka (36%), Kidal (29%), Gao (25%), Tombouctou (23%), and Taoudenit (18%)—had the highest proportions of presumed malaria cases among the total. In the center and south, presumed cases contributed to only 4% of malaria cases in the Mopti region and up to 15% in the regions of Bamako and Sikasso. Overall, 8% and 12% of malaria cases reported by CHW and health center personnel, respectively, were presumed cases. On average, in 2019, only 5% of health facilities reported stockouts of rapid diagnostic tests (RDTs) while 10% reported a stockout of any artemisinin-based combination therapy (ACT)—chiefly adult formulations (20%). All the regions in Mali have personnel trained in malaria case management. However, for many reasons, healthcare workers continue to treat patients with negative diagnostic test or no test. Our analysis shows that regular monitoring of reported presumed malaria cases can be useful in informing programs on potential stockouts of RDTs and in identifying health facilities needing supportive supervision to improve malaria case management.

661

EVALUATION OF THE Q-PLEX™ HUMAN MALARIA ARRAY FOR THE DETECTION OF *PLASMODIUM KNOWLESI*

Rebecca Barney[1], Robert Moon[2], Andrew Rashid[1], Ihn Kyung Jang[1], Gonzalo J. Domingo[1]

[1]*PATH, Seattle, WA, United States,* [2]*London School of Hygiene & Tropical Medicine, London, United Kingdom*

Plasmodium knowlesi is a parasitic species originally found in macaques that can also cause human malaria infections throughout Southeast Asia. There is a necessity for low-cost, easy-to-use assays for detection of *Plasmodium knowlesi*. PATH previously demonstrated the utility of the multiplex malaria enzyme-linked immunosorbent assay, commercialized as the Q-Plex™ Human Malaria Array (5-plex; Quansys Biosciences), which can detect malaria antigens including *P. falciparum*–specific histidine-rich protein 2 (HRP2), Pan-malaria lactate dehydrogenase (panLDH), *P. falciparum*–specific LDH (*Pf* LDH), *P. vivax*–specific LDH (*Pv* LDH), and human C-reactive protein in development of next-generation HRP2 or *Plasmodium* LDH-based rapid diagnostic tests (RDTs). *P. knowlesi*–specific LDH (*Pk* LDH) exhibits 98.6% homology to *Pv* LDH and has been shown to cross-react with monoclonal antibodies to *Pv* LDH. As such we hypothesize that *Pk* LDH may be detectable by the *Pv* LDH and panLDH specific array spots in the Q-Plex array. In this study, we aimed to evaluate the capability of the Q-Plex array as well as a variety of commercially available RDTs in detecting LDH protein from *P. knowlesi* parasites. Red blood cell pellets and supernatants from highly synchronized *P. knowlesi* culture were collected over one life cycle. *Pk* LDH was assayed for reactivity against panLDH and *Pv* LDH antibodies by Q-Plex array. Parasite stage and percent parasitemia were determined via microscopy. *Pv* LDH and panLDH detectors in the Q-Plex array were reactive to *Pk* LDH. Minimal to no cross-reactivity was noted with the *Pf* LDH and HRP2 assays. Of note, we found variable reactivity of pLDH from *P. knowlesi* culture with pLDH-based RDTs. In conclusion, our results demonstrate promising performance characteristics of the Q-Plex array for the accurate detection of *P. knowlesi* infections, but further investigation into *P. knowlesi* cross-reactivity with pLDH-based RDTs is of interest.

662

MALARIA ANTIGEN PROFILING TO ASSESS RAPID DIAGNOSTIC TEST PERFORMANCE AND STAGE OF INFECTION IN KINSHASA PROVINCE, DEMOCRATIC REPUBLIC OF THE CONGO

Alpha Oumar Diallo[1], Melchior Kashamuka Mwandagalirwa[2], Kristin Banek[3], Joseph A. Bala[2], Marthe Nkalani[2], Georges Kihuma[2], Tommey Nseka Mambulu[2], Joseph Atibu[2], Georges Emo Mahilu[2], Lauren Mccormick[1], Kyaw L. Thwai[1], Jonathan J. Juliano[3], Steven R. Meshnick[3], Antoinette Kitoto Tshefu[1], Jonathan B. Parr[3]

[1]*Department of Epidemiology, University of North Carolina-Chapel Hill, Chapel Hill, NC, United States,* [2]*Ecole de Santé Publique, Faculté de*

Médecine, University of Kinshasa, Kinshasa, Democratic Republic of the Congo, [3]Institute for Global Health and Infectious Diseases, University of North Carolina-Chapel Hill, Chapel Hill, NC, United States

The Democratic Republic of the Congo (DRC) has the second highest malaria burden worldwide. To understand how human and mosquito factors affect malaria transmission and to assess malaria diagnostic performance, we are conducting a prospective longitudinal study in three health areas with varying endemicity in Kinshasa Province. We describe characteristics of participants from the baseline household survey conducted in March 2018 during which we completed questionnaires, tested participants for malaria via rapid diagnostic tests (RDTs) and collected dried blood spots for polymerase chain reaction (PCR) testing and antigen testing using a bead-based multiplex immunoassay (Luminex). We evaluate RDT performance compared to PCR and Luminex detecting parasite lactate dehydrogenase (pLDH), aldolase, and *Plasmodium falciparum* histidine-rich protein 2 (HRP2). In addition, we classify *P. falciparum* (Pf) infection status using Luminex antigen profiles and evaluate associations with demographic and clinical profiles. 1,538 participants living in 239 households were enrolled, the majority of whom (72%) reside in rural areas. Participants of all ages were enrolled, with a median age of 15 years. Participants were 34%, 34%, and 49% positive by RDT, PCR, and Luminex, respectively, during initial analysis. HRP2-based RDTs were 65% sensitive and 87% specific compared to *P. falciparum*-specific *pfldh* PCR. Compared to Luminex, RDTs were 60% sensitive and 88% specific for HRP2. The prevalence of active Pf infection (defined as positive for HRP2 and ≥1 other antigen by Luminex) was 40% among all participants. Active Pf infection prevalence was 53% among children aged 5-14 years old and 56% among those reporting fever in the last seven days. Multivariate analyses are underway to identify associations between antigen profile and patient characteristics. These baseline results, along with future analyses of follow-up data that incorporate household characteristics with entomological data, will elucidate factors associated with malaria prevalence, infection status, and transmission across varying endemicity levels in Kinshasa.

663

USING GAZELLE HEMOZOIN BASED MALARIA DIAGNOSTIC TO DIFFERENTIATE BETWEEN *PLASMODIUM* SPECIES

Praveen K. Bharti[1], Madhav Patel[1], Anil Kumar Verma[1], Bina Srivastva[2], Shweta Srivas[1], Digvijay Sah[1], Priyaleela Thota[3], Tyler Witte[3], Anne Rocheleau[3], Aparup Das[1], Anup Kumar Anvikar[2]

[1]*ICMR - National Institute of Research in Tribal Health, Jabalpur, India,* [2]*ICMR - National Institute of Malaria Research, New Delhi, India,* [3]*Hemex Health, Portland, OR, United States*

Malaria is caused by a family of *Plasmodium* parasites, five of which infect humans. *P. falciparum* has the highest mortality rate of the species, and *P. vivax* subjects the patient to risk of relapse. The infecting species can impact clinical treatment giving a strong impetus to differentiate between species. Gazelle™ is a platform technology that presents a unique opportunity for fast (~one minute), easy, and accurate diagnosis of malaria. The system detects the presence of hemozoin, which is a paramagnetic crystal that is a waste product of all malaria species. A diluted blood sample is placed in an alternating high and low magnetic field. Any hemozoin present align in the high magnetic field and randomize due to Brownian motion in a low magnetic field. The amount of alignment is measured with light since aligned hemozoin absorbs more light. The signal is analyzed by an algorithm with the results presented on-screen so there is no interpretation necessary by the user. The various species of malaria have different sizes of hemozoin. The randomization time is longer for species such as *P. falciparum,* with large hemozoin crystals, compared with the *P. vivax* which has much shorter hemozoin. A large study of 6500 subjects is underway across six sites in India with the goal of differentiating *P. falciparum* from other species. These sites include three National Institute of Research in Tribal Health sites and three National Institute of Malaria Research sites. Results from the first 1500 subjects will be presented at the conference, but early analysis indicates that the accuracy of differentiation of *P. falciparum* from other species is 95%.

664

OUTCOME OF MALARIA RAPID DIAGNOSTIC TEST SCALE UP ON REDUCING PRESUMPTIVE DIAGNOSIS OF MALARIA IN CHALLENGING HEALTH SETTINGS: EVIDENCE FROM 8 NIGERIAN STATES

Chidinma Assumpta Okolo[1], Victoria Erinle[2], Frank Oronsaye[1], Adeyoju Temitope[1], Uchechukwu Ikhimioya[1], Ghislain Ismael Nana[1], Nnaemeka onugu[1], Ezinne Onuba[1], Emmanuel Shekarau[3], Olatayo Abikoye[1], Ibrahim Maikore[3], Diwe Ekweremadu[1], Sonachi Ezeiru[1]

[1]*Catholic Relief Services, Abuja, Nigeria,* [2]*Management Sciences for Health, Abuja, Nigeria,* [3]*National Malaria Elimination Programme, Federal Ministry of Health, Abuja, Abuja, Nigeria*

The nigerian national malaria treatment guidelines recommends all fever cases should be tested before treatment with Artemisinin-based combination therapy (ACTs) in line with WHO recommendation. However, presumptive diagnosis of malaria is widely practiced in Nigeria especially where diagnosis is not accessible thus influencing health workers to resort to presumptive diagnosis of malaria. The national Malaria Elimination programme has been supported by donors like Global Fund(GF) through Catholic Relief Services to implement the national malaria guideline through provision of malaria RDT kits, training of health care workers on malaria case management, activities to change provider behaviour, and provide supervision in public health facilities across 24 States in 2017 and 13 States in 2018-2020. The aim of this study is to assess and compare the outcome of the scale-up of RDT on reducing presumptive diagnosis of malaria in 4 selected States with interventions from GF (Kaduna, Kwara, Niger and Osun) and 4 States without any interventions from donors(control) (Bayelsa, Abia, FCT and Lagos). A desk review was conducted to calculate proportion of key diagnostic indictors from DHIS2 database for 8 states in 2016 as baseline and 2019 as endline result. In 2016, the average RDT uptake was 59% in control States and 79% in the intervention states. This decreased to 34% in the control and increased to 95% in the intervention states in 2019. Average microscopy uptake in 2016 was 21% in control and 4% in intervention states. It increased to 34% in the control states and decreased to 2% in the intervention states in 2019. Comparatively, there was a marked decrease in presumptive diagnosis of malaria from 60%, 22%, 21% & 29% in 2016 to 1%, 2%, 3% & 3% in 2019 in Kaduna, Kwara, Niger and Osun States respectively. While there is still a high level of presumptive diagnosis of malaria practiced in the control States from 77%, 46%, 56%, 31% in 2016 to 39%, 57%, 48% & 34% in 2019 in Bayelsa, Abia, FCT and Lagos States respectively. This suggests that improved access to and use of malaria RDT kits by healthcare workers may contribute in reducing presumptive diagnosis of malaria.

665

DIFFERENCES IN RDT PERFORMANCE IN ACTIVE VERSUS PASSIVE MALARIA SURVEILLANCE IN KINSHASA PROVINCE, DEMOCRATIC REPUBLIC OF CONGO

Nono Mazangama Mvuama[1], Melchior Mwandagalirwa Kashamuka[1], Joseph A. Bala[1], Georges Kihuma[1], Joseph Atibu[1], Marthe Nkalani[1], Chris Hennelly[2], Kyaw L. Thwai[2], Aimé Lumaka[3], Margaret Carrel[4], Jonathan J. Juliano[5], Antoinette Kitoto Tshefu[1], Steve R. Meshnick[5], Jonathan B. Parr[2]

[1]*Kinshasa School of Public Health, School of Medicine, University of Kinshasa, Kinshasa, Democratic Republic of the Congo,* [2]*Institute for Global Health and Infectious Diseases, University of North Carolina-Chapel Hill, Chapel Hill, NC, United States,* [3]*Center for Human Genetics, School of Medicine, University of Kinshasa, Kinshasa, Democratic Republic of the Congo,* [4]*Department of Geographical and Sustainability Sciences,*

University of Iowa, Iowa City, IA, United States, [5]Institute for Global Health and Infectious Diseases, University of North Carolina-Chapel Hill; Department of Epidemiology, University of North Carolina-Chapel Hill, Chapel Hill, NC, United States

For many low-income regions like the Democratic Republic of Congo, rapid diagnostic tests (RDTs) are the most common and often the only available tool for malaria diagnosis. We evaluated the performance of RDTs as part of a longitudinal study of malaria transmission in one urban and six rural sites in two health zones in Kinshasa. 1,591 participants from 242 households were enrolled in the study. We evaluated RDT (SD Bioline Malaria Ag Pf/Pan) performance during twice yearly cross-sectional study visits (active surveillance) and when symptomatic participants sought care at the study health centers (passive surveillance) using a series of PCR assays, a Luminex bead-based immunoassay for *P. falciparum* histidine-rich protein 2 (HRP2), and Sanger sequencing. From 2015 to 2018, 8,791 RDTs were performed and 8,710 samples subjected to PCR. 46% of RDT and 43% PCR results were positive for *Plasmodium falciparum*. Agreement between RDT and PCR results was moderate (kappa=0.58) overall, but stronger during active than passive surveillance (kappa=0.62 *vs.* 0.36, respectively). Prevalence also varied by type of encounter, with 29% RDT and 35% PCR prevalence during active surveillance and 79% RDT and 59% PCR prevalence during passive surveillance. The odds of having discordant RDT and PCR results were highest in passive surveillance (OR: 2.15; CI 1.93-2.39), subjects ≥25 years old (OR: 1.19; CI 1.02-1.38; <5 years reference) and in rural sites (OR: 4.25, CI 3.51-5.16) in univariate analysis. PCR and Luminex confirmed the presence of intact *pfhrp2/3* genes and HRP2 antigenemia, respectively, in all RDT-negative/PCR-positive samples tested. Analysis of *pfhrp2/3* genetic diversity by Sanger sequencing is underway. In conclusion, RDT performance compared to PCR varied by surveillance strategy, as well as age and location. Concordance between RDT and PCR results was highest during active surveillance visits. Discordance during passive surveillance is likely driven by lingering HRP2 antigenemia after recent clearance of parasitemia. Regular evaluation of RDT performance in the field is needed to inform research and support malaria control interventions.

666

ANTIBIOTICS OVERUSE AND VARYING RATES OF MALARIA TESTING IN CAMBODIA BASED ON ROUTINE PATIENT REGISTERS IN PRIMARY HEALTH CENTERS

Siv Sovannaroth[1], Pengby Ngor[1], Sabine Dittrich[2], Sandra Incardona[2]

[1]*National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia,* [2]*FIND, Geneva, Switzerland*

The management of febrile patients is a major problem in areas with little access to good quality diagnostics. If a malaria test is negative, there is no readily available test to differentiate bacterial from non-bacterial infections to guide antibiotics prescription. Yet a rationale use of antibiotics is critical to avoid further spread of antimicrobial resistance (AMR). A recent review of published AMR studies in Cambodia has reported high AMR rates such as 92.8% of *E. coli* isolates being resistant to ampicillin. A study was conducted to better understand routine testing and treatment practices of health workers (HWs) in public health centers (HCs). Antibiotic and antimalarial prescription as well as malaria RDT (mRDT) testing data were collected in twelve HCs of Kampong Speu from 2019. This province is located West to the country's capital and had a mRDT positivity rate of 16% in 2019. Data were transferred from HCs' routine patient registers into an online database on tablets for subsequent analysis. Preliminary results showed that only 10.8% of patients with documented or self-reported fever were tested with a mRDT, with testing rates being higher (32.7%) in HCs that report more than 40 confirmed malaria cases annually, versus the others (1.8%). A high proportion of febrile cases (75%) were treated with an antibiotic drug. Among mRDT positive patients, 99% and 8% received an antimalarial and an antibiotic treatment, respectively. The antibiotic prescription rate among mRDT negative patients was 78%, and only 0.25% received an antimalarial. Previously published reports have shown that antibiotics use in Cambodia is widespread and weakly controlled, especially among informal health care providers and drug sellers, however this is the first report documenting routine antibiotic use in public health facilities. The results also demonstrate that antimalarial prescription is well in line with mRDT results, although testing rates are surprisingly low. The data collection process and full results will be presented, along with a discussion of possible factors explaining the observations and of ways to achieve a better targeted antibiotics use.

667

PREVALENCE OF *PLASMODIUM FALCIPARUM* ISOLATES LACKING HISTIDINE RICH PROTEIN 2 AMONG SYMPTOMATIC PATIENTS IN KWILU PROVINCE (DR. CONGO)

Yannick Bazitama Munyeku[1], Alain Abera Musaka[2], Richard Leighton Culleton[3], Christopher Smith[3], Paul Mankadi Mansiangi[4]

[1]*National Division of Health Laboratories. DR Congo, Kinshasa, Democratic Republic of the Congo,* [2]*Kwilu Provincial Health Division. DR Congo, Kikwit, Democratic Republic of the Congo,* [3]*Nagasaki University, Graduate School of Tropical Medicine and Global Health, Nagasaki, Japan,* [4]*University of Kinshasa, School of Public Health, Kinshasa, Democratic Republic of the Congo*

Malaria rapid diagnostic tests have become a primary and critical tool for malaria diagnosis in malaria-endemic countries where *Pf*HRP2-based RDTs are widely used. Since about a decade, *Pf*HRP2-based RDTs' accuracy is challenged by isolates harbouring *pf*hrp2 gene deletion, causing false-negative results. In DR. Congo, few is known about the prevalence of *pf*hrp2 gene deletion among symptomatic patients, especially in low to moderate transmission areas where isolates with *pf*hrp2 deletion are assumed to emerge and spread. This study aimed to determine the local prevalence of the *pf*hrp2 gene deletion among malaria symptomatic patients and associated factors in the Kwilu province, a low to moderate malaria transmission area. We used secondary data from a prospective cross-sectional study conducted on 684 individuals of all ages, seeking healthcare from October to December 2018 in 34 randomly selected health facilities in the Kwilu province, for symptoms suggestive of malaria. Data were collected using a structured questionnaire. Blood was collected for microscopy, applied on *Pf*HRP2-RDT, and spotted on Whatman filter paper to prepare DBS. Genomic DNA was extracted from membranes of spent *Pf*HRP2-RDT cassettes and DBS. For the *pf*hrp2 gene detection, we performed a nested PCR assay targeting a 228 bp fragment spanning from exon 1 to a portion of exon 2 of *pf*hrp2 gene. To confirm *Plasmodium falciparum* infection and *pf*hrp2 gene deletion, we performed a real-time PCR assay targeting a 226 bp region of the *P. falciparum* lactate dehydrogenase. Data were analyzed using STATA15. Fischer's exact test and the Kruskal-Wallis test were applied with a level of statistical significance set at $p < 0.05$. The overall prevalence of *pf*hrp2 gene deletion was 9.2%. Deletion of *pf*hrp2 gene was associated with health zone of origin (p=0.012) and age (p=0.019). Among false-negative *Pf*HRP2-RDT results, only 9.9% were due to *pf*hrp2 gene deletion. *Plasmodium falciparum* isolates with *pf*hrp2 gene deletion are common among symptomatic patients in Kwilu province. The use of RDTs targeting both *Pf*HRP2 and pLDH antigens could limit the spread of deleted isolates.

668

CONTRIBUTION OF *PLASMODIUM FALCIPARUM* PARASITES WITH *PFHRP2* GENE DELETIONS TO FALSE NEGATIVE HRP2-BASED MALARIA RDT RESULTS IN GHANA: A NATIONWIDE STUDY

Linda Eva Amoah[1], Ben Abuaku[1], Dickson Donu[1], Agbana H. Bukari[1], Eunice O. Amoako[1], George A. Asumah[2], Alexander Asamoah[2], Nana Yaw Preprah[2], Eric Rogier[3], Michael Aidoo[3], Keziah Malm[2]

[1]*Noguchi Memorial Institute for Medical Research - University of Ghana, Accra, Ghana,* [2]*Ghana National Malaria Control Program, Accra, Ghana,*

[3]*Centers for Disease Control and Prevention, Center for Global Health, Division of Parasitic Diseases and Malaria, Malaria Branch, Atlanta, GA, United States*

False-negative malaria rapid diagnostic test (RDT) results amongst symptomatic malaria patients are detrimental as they could lead to ineffective malaria case management. This study determined the nationwide contribution of parasites with deletions in the *Pfhrp*2/3 genes to false negative RDT results in Ghana. Whole blood was collected from volunteers presenting with malaria symptoms at 100 health facilities across the country from May to August 2018. Thick and thin blood smears, dried blood spots as well as an HRP2 RDT kit were prepared for each sample. Genomic DNA was extracted from the cell pellets and or dried paper blots. Species-specific *18S rRNA* PCR, merozoite surface protein (MSP1) and glutamate rich protein (GLURP) PCR were used to identify *Plasmodium falciparum* positive samples, which were subsequently subjected to *Pfhrp*2/3 genotyping. Out of the 19,787 patients enrolled, 67.7% (13,135/19,402) were negative by RDT and 15.5% (2,890/18,618) were positive by microscopy. There were 4.7% (136/2,890) false negative RDT results of which 68.1% (79/116) tested positive by *P. falciparum* as well as MSP1 and GLURP PCR. Genotyping of exon 1-2 and exon 2 of the *Pfhrp*2 gene identified 12.8% (10/78) and 39.5% (31/79) of samples respectively to have deletions. Genotyping exon 1-2 and exon 2 of the *Pfhrp*3 gene identified 15.2% (12/79) and 41.0% (32/78) of samples respectively to have deletions. Only 5% (4/79) samples had deletions in both exon 1-2 and exon 2 of the *Pfhrp 2 gene*. A national prevalence of parasites with deletions in exon 2 of the *Pfhrp*2 amongst all the microscopy positive symptomatic malaria patients was 1.1% (31/2,890). This study demonstrates that HRP2 based malaria RDTs remain effective in diagnosing symptomatic malaria patients across all the Regions of Ghana.

669

DISCOVERY OF CANDIDATE SALIVA BIOMARKERS OF *PLASMODIUM VIVAX* INFECTIONS

Borja López-Gutiérrez[1], Timothy Hamerly[1], Greys Braga[2], Hugo Valdivia[2], Leonila Ricopa[2], Dannet Bishop[2], Rhoel R. Dinglasan[1]

[1]*Department of Infectious Diseases and Immunology, Emerging Pathogens Institute, College of Veterinary Medicine, University of Florida, Gainesville, FL, United States,* [2]*U.S. Naval Medical Research Unit No. 6, Bellavista, Callao, Peru*

The large expansion of malaria control interventions experienced during the last two decades have resulted in a remarkable reduction in the number of cases and deaths attributed to malaria. While most of these interventions are geared towards *Plasmodium falciparum*, the often-neglected *P. vivax* is the most geographically widespread species and the leading cause of malaria outside Africa. However, the distinctive biology of *P. vivax* underpins the urgent need to develop novel control measures that tackle the features that render *P. vivax* less sensitive to the current interventions. In this regard, diagnosis of *P. vivax* is hampered by the ability of the parasite to remain dormant in the liver, lower bloodstream parasite densities that may remain undetected by light microscopy and pan-specific rapid diagnostic tests, or a rapid emergence of infectious gametocytes (thus increasing the likelihood of a transmission event before the onset of symptoms). The proteomic analysis of saliva from asymptomatic individuals infected by *P. falciparum* led to the identification of >30 parasite proteins, from which *Plasmodium* sexual stage protein 17 (PSSP17, PF3D7_1218800) was further validated as a promising biomarker for the diagnosis of submicroscopic gametocyte carriage, and the development of a saliva-based lateral flow immunoassay rapid test. Considering the contrasting developmental biology of *P. falciparum* and *P. vivax*, it is unclear if PSSP17 would be a relevant biomarker for vivax malaria. Clearly, the identification of asexual and/or gametocyte proteins in the saliva of individuals infected with low *P. vivax* parasitemias could lead to the development of a *P. vivax*-specific diagnostic test for the prompt diagnosis of infected individuals that contribute to the transmission of the parasite. To do so, we collected saliva and matched blood samples for qPCR analysis from 43 individuals infected with *P. vivax* from Peru. The mass spectrometry-based proteomic profiling of pooled saliva allowed for the identification of potential biomarkers matched against estimated sub-microscopic parasite density estimates by qPCR for the early diagnosis of *P. vivax*.

670

COMPARISON OF TWO MALARIA MULTIPLEX REFERENCE IMMUNOASSAYS IN THE MEASUREMENT OF MALARIA ANTIGENS

Ihn Kyung Jang[1], Alfons Jimenez[2], Andrew Rashid[1], Rebecca Barney[1], Allison Golden[1], Xavier C. Ding[3], Alfredo Mayor[2], Gonzalo J. Domingo[1]

[1]*PATH, Seattle, WA, United States,* [2]*The Barcelona Institute for Global Health, Barcelona, Spain,* [3]*Foundation for Innovative New Diagnostics, Geneva, Switzerland*

Different malaria reference immunoassays have been used to simultaneously measure concentration of malaria antigens by several laboratories in order to, facilitate the evaluation for next-generation rapid diagnostic tests. Understanding the inter-platform variability will enable comparison of data generated by the different platforms. A panel of recombinant antigens, International standard, and clinical specimens prepared in the forms of blood pellet and dried blood spot was developed to characterize the performance of different quantitative malaria antigen detection platforms. These were tested on the Quansys Biosciences Q-Plex™ and Luminex multiplex reference immunoassays. Here we present the evaluation of both assays for variability; reactivity to histidine-rich protein 2 (HRP2) and HRP3; detection of parasites with *hrp2/hrp3* deletion; detection of non-*Plasmodium falciparum* and non-*Plasmodium vivax* species; and reactivity of lactate dehydrogenase proteins from difference sources. The results from this study should facilitate the comparison and correlation of data generated by two different immunoassay platforms.

671

MOLECULAR DETECTION OF *PLASMODIUM* IN AUTOCHTHONOUS MALARIA AREAS LOCATED IN SAO PAULO STATE ATLANTIC FOREST BIOME

Mariana Aschar[1], Maria L. Farinas[1], Maria J. Costa-Nascimento[2], Silvia M. Di Santi[3]

[1]*Sao Paulo University, Sao Paulo, Brazil,* [2]*SUCEN, Sao Paulo, Brazil,* [3]*Sao Paulo University, São Paulo, Brazil*

Malaria is an infectious disease transmitted by *Anopheles* infected by *Plasmodium* and is still a challenge for science and control programs. In Brazil, most cases occur in endemic area, the Amazon Region. However, in the Extra-Amazon Region, considered as a non-endemic area, autochthonous cases are reported, presenting low parasitemias and mild symptoms. The state of São Paulo has historically presented malaria transmission in regions located in the Atlantic Forest Biome. The number of cases reported by the surveillance agencies can be underreported, since the diagnosis is based on the reference test, the thick blood smear (TBS), whose sensitivity varies significantly depending on the observer's skill. In models using TBS as reference, it is known that submicroscopic infections are less observed in areas of high transmission, increasing to high levels in areas of low transmission intensity. The occurrence of asymptomatic infections has an impact on malaria control and elimination programs. Molecular tests show high sensitivity and are useful for detecting asymptomatic individuals with low parasitemias. The aim of this study was to evaluate the prevalence of individuals carrying *Plasmodium* in inhabitants from autochthonous malaria areas in the state of Sao Paulo, Brazil. The methods used were the microscopy, the gold standard for the control program, compared with a high sensitive qPCR. We processed 348 samples by both protocols. Results revealed difference statistically significant in positivity between TBS (4.89%) and qPCR (17.53%), p= 0,51. Poor association was observed between positivity in TBS compared to qPCR (κ=0.0028). Considering the high sensitivity and specificity of the qPCR used here, it is possible to conclude that TBS presented false positive

and negative results. The use of molecular protocols in the population living in areas adjacent to autochthonous cases will allow the detection of asymptomatic individuals harboring *Plasmodium*, detecting sources for transmission of new cases and improving the surveillance and control activities.

672

ACCESS TO MALARIA RAPID DIAGNOSTIC TESTS AND HEALTHCARE PROVIDERS' KNOWLEDGE OF, AND STORAGE PRACTICES IN PUBLIC HEALTH FACILITIES IN NIGERIA

Sonachi Ezeiru[1], Diwe Ekweremadu[1], Temitope Ipinmoye[2], Genevieve Eke[3], Victor Adebayo[2], Olatayo Abikoye[1], Ezinne Onuba[2], Chukwudi Uche[3], Wellington A. Oyibo[4], Bala Audu[5]

[1]*Catholic Relief Services (CRS)(Principal Recipient of the Global Fund, Malaria in Nigeria), Federal Capital Territory, Abuja, Nigeria,* [2]*Catholic Relief Services (CRS)(Principal Recipient of the Global Fund, Malaria in Nigeria), Federal Capital Territory, Lagos, Nigeria,* [3]*Independent Consultant, Abuja, Nigeria,* [4]*ANDI Centre of excellence for Malaria Diagnosis, College of Medicine of the University of Lagos, Lagos, Nigeria,* [5]*National Malaria Control Programme, Federal Ministry of Health, Abuja, Nigeria*

Parasitological confirmation of suspected cases of malaria using microscopy or malaria rapid diagnostic tests (RDTs) is the current best practices in the case management of malaria. Malaria RDTs do not require the huge infrastructure and trained personnel for microscopy and is therefore useful in providing access to testing. RDTs are deployed in public health facilities (HFs) and by the National Malaria Strategic Plan (NMSP, 2014-2020), by 2020 all suspected cases (100%) should be tested before treatment. Access to testing and adequate storage of RDT is critical to the implementation of this policy and target attainment. This study was conducted in public health facilities in three Global Fund-supported states of Nigeria, namely, Delta 122(36.3%), Kaduna 149 (44.3%) and Ogun state (63 (19.4%) where RDT post-deployment surveillance was conducted in 2018. The healthcare providers (HCPs) that responded were from General Hospital (16.1%), PHCs (80.4%), Dispensary (1.0%), National Medical Store (0.5%), State Medical Stores (2.0%) and tertiary intuitions. RDT was reported to be available or being used in 90% of the HFs visited and were provided free and where it was paid for, the cost was between N200 - N500 ($0.55 - $1.39). Our assessment of HCPs knowledge and appropriateness of storage practices showed that only about 50% knew the manufacturer's storage requirement for malaria RDTs while RDTs were inappropriately stored in 35% of the HFs. The sources of malaria RDTs were mainly through donor-funded programmes and distributed by Partners such as the Catholic Relief Services (CRS). In few cases, HFs procured RDTs from the private sector and these were the RDTs that were paid for by the clients. Of the 334 HFs in the three states, only 7.5% had thermometer where RDTs were stored. Availability of malaria RDTs in HFs should be aggressively promoted since no additional infrastructure is needed for effective malaria case management as well as meeting set national target. Training modules for HCPs should emphasize appropriate RDT storage and temperature monitoring.

673

MICROSATELLITE GENOTYPING OF *PLASMODIUM VIVAX* MALARIA CASES IN NEPAL

Komal R. Rijal

Tribhuvan University, Kathmandu, Nepal

Plasmodium vivax is the main cause of malaria in Nepal. Relapse patterns have not been characterized previously. Radical treatment with primaquine to prevent relapse is widely recommended but often not given. Patients with acute *P. vivax* malaria were randomized to receive chloroquine (CQ: 25 mg base/kg given over 3 days) alone or together with primaquine (CQ+PQ: 0.25 mg base/kg/day for 14 days). Patients were followed intensively for one month then at 1-2-month intervals for one year. Parasite isolates were genotyped using 9 polymorphic microsatellite markers to assess the genetic relatedness of recurrent malaria episodes. 101 (49%) patients received CQ and 105 (51%) received CQ+PQ. By day 3 95% of patients in the CQ+PQ arm had cleared parasitemia versus 83% in the CQ arm; p<0.005. In the CQ+PQ arm there were 3 (4.1%) recurrences in the 73 patients who completed one-year follow-up compared with 22 (28.2%) of 78 in the CQ only arm; risk ratio (95% CI) 0.146 (0.046 to 0.467): p<0.0001. Microsatellite genotyping showed relatively high *P. vivax* genetic diversity; mean He 0.843 (He 0.570 to 0.989) with low multiplicity of infection (mean MOI: 1.05) reflecting a low transmission pre-elimination setting. Of the 12 genetically homologous relapses 5 (42%) occurred in a cluster after 9 months indicating long latency. Although there may be emerging chloroquine resistance, the combination of chloroquine and the standard dose 14-day primaquine regimen is highly efficacious in providing radical cure of short and long latency *P. vivax* malaria in Nepal.

674

IVERCURE - A DOSE-ASCENDING, RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE-BLIND CLINICAL TRIAL ON THE EFFICACY AND SAFETY OF IVERMECTIN FOR THE TREATMENT OF *PLASMODIUM FALCIPARUM* INFECTIONS IN ASYMPTOMATIC GABONESE ADULTS: PRELIMINARY RESULTS

Dorothea Sträßner[1], Rella Zoleko Manego[2], Jana Held[3], Benjamin Mordmüller[3], Laura C. Kalkman[2], Ayola A. Adegnika[2], Michael Ramharter[1], Ghyslain Mombo-Ngoma[2]

[1]*Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany,* [2]*Centre de Recherches Médicales de Lambaréné, Lambaréné, Gabon,* [3]*Institute of Tropical Medicine, University of Tübingen, Tübingen, Germany*

Malaria remains a relevant health hazard. Recently, the endectocide Ivermectin has been considered as a new complementary malaria vector tool for controlling and eliminating the disease by 2030. Experiences from ivermectin *in vitro* data promise a good parasiticidal effect. Still, there is a need for reliable *in vivo* data to account for a therapeutic benefit before this preventive indication can be implemented. We are conducting a clinical trial evaluating safety and efficacy of different ivermectin doses in asymptomatic adults with *Plasmodium falciparum* infection in Gabon. Volunteers recruited are adults, aged ≥18 years with an asymptomatic *P. falciparum* infection of 200 to 5000 Pf/µl , and no *Loa loa* detected by microscopy. This is a Phase 1, dose-ascending, double-blind, randomized, placebo-controlled trial. A total of 49 participants were assigned to four groups. The first three groups of five subjects each served to cautiously escalate dose and duration of the treatment with one to three days of 200µg/kg ivermectin. The fourth group of 34 subjects is the randomized part of the study with subjects receiving three doses of either placebo or ivermectin at 300 µg/kg. All participants were hospitalized for 3 days with an active follow-up period of 7 days. *Plasmodium* parasitaemia was measured each eight hours during hospitalisation and each day up to end of active follow-up. Participants recruited in all cohorts had a median age of 25 years (IQR 20.5-49.0), sex ratio of 1.8 and a median baseline parasite density of 503 Pf/µl (IQR 296-982). In all treatment arms, parasitaemia levels decreased following the administration of ivermectin from 1133 Pf/µl to 691 Pf/µl by 32 hours. In the randomized cohort, 75% (12/16) participants showed at least two consecutive slides with <10% of baseline parasitaemia within a week. The mean parasite clearance time to <100 *Pf*/µl was 26h and 75% (12/16) of participants remained cleared from on average 141h post-treatment on. There have been 34 Adverse Events, of which none was serious. The most frequent AEs were headache, skin rash and fever. More detailed results of the respective treatment arms will be presented at the meeting.

677

COMPARATIVE EFFICACY AND SAFETY OF PYRONARIDINE-ARTESUNATE VERSUS ARTEMETHER-LUMEFANTRINE IN THE TREATMENT OF ACUTE UNCOMPLICATED MALARIA AMONG CHILDREN IN SOUTHWEST NIGERIA-AN INTERIM REPORT

Catherine Olufunke Falade[1], Obaro Stanley Michael[1], Fiyinfoluwa Ibukun Olusola[1], Roland Ibenipere Funwei[1], Abiola Lukman Olusanya[1], Oluwafunmibi Enitan Anjorin[2], Bosede O. Orimadegun[1], Aduragbenro D. Adedapo[1], Sumi Oh[3], Babatunde Aderemi Aderele[3], Rene Cazetien[3], Jangsik Shin[3]

[1]*University of Ibadan, Ibadan Nigeria, Ibadan, Nigeria,* [2]*Babcock University, Ilisan-Remo, Nigeria,* [3]*Shin Poong Pharmaceuticals, South Korea, Seoul, Republic of Korea*

Artemether-lumefantrine (AL) has been the ACT of choice in Nigeria since 2005. Declining responsiveness of childhood *Plasmodium falciparum* infections to AL in Nigeria has been reported. Pyronaridine-artesunate (PA) is a novel fixed-dose ACT indicated for the treatment of uncomplicated *falciparum* and *vivax* malaria in adults and children. In an open randomized clinical trial, we compared the efficacy and safety of AL and PA using the 28 day WHO antimalarial efficacy protocol. Patients received at standard dosage according to body weight either AL twice a day (Coartem® dispersible tablets, Novartis pharma) or PA once a day (Pyramax® tablets or granules, Shin Poong Pharmaceuticals). Capillary blood samples were obtained for thick blood smears, hematocrit and filter paper samples from enrollees at all contact times. Venous blood was obtained for hematology, blood chemistry and liver function tests at intervals as part of safety evaluation. 130/137 (94,8%) of enrollees aged three months to 14 years completed the study. 63 (48.5%) received AL while 67 (51.5%) received PA. Geometric mean parasite densities were 30,967/µL and 25,493/µL in AL and PA groups respectively (ρ=0.978). PCR-uncorrected ACPR at D28 of PPP was 71.4%(45/63) for AL and 80.6%(60/67) for PA (ρ=0.008). PCR-corrected ACPR was 96.8 for AL and 100% for PA [ρ=0.233]. PA recorded a significantly better PCR-uncorrected ACPR than AL among children <60 months of age [95.5%;21/22 versus 55%;11/20 (ρ =0.008)]. An enrollee who received AL failed treatment on D14. Although hematological recovery was good in both treatment arms, mean Day 28 hematocrit was significantly higher for PA versus AL [34.78%±2.83 versus 33.31%±3.68 (ρ=0.026)]. The safety profile of both drugs was good. There was no clinical evidence of jaundice, intravascular hemolysis, hepatic dysfunction or renal impairment. Blood chemistry and liver function tests ware mostly within normal limits with occasional marginal rise. In conclusion, AL and AP are safe and efficacious for the treatment of uncomplicated malaria in Southwest Nigeria. However, post treatment prophylactic effect of PA is better than AL.

678

FIRST-IN-HUMAN EVALUATION OF A *PLASMODIUM FALCIPARUM* TRANSMISSION-BLOCKING MONOCLONAL ANTIBODY

Saskia C. van der Boor[1], Merel J. Smit[1], Yimin Wu[2], Teun Bousema[1], Matthijs M. Jore[1], Christian F. Ockenhouse[2], Robert W. Sauerwein[1], Matthew B. McCall[1]

[1]*Radboudumc, Nijmegen, Netherlands,* [2]*PATH Malaria Vaccine Initiative, Washington D.C., DC, United States*

Novel approaches are urgently required to help control the global burden of malaria, including transmission-blocking interventions. TB31F is a humanized version of monoclonal antibody (mAb) 45.1, the most potent transmission-blocking mAb for *Plasmodium falciparum* described to date, which recognises the Pfs48/45 protein on male *P. falciparum* gametocytes and early gametes and inhibits their fertilisation of female gametes. Uptake of TB31F by blood-feeding mosquitoes prevents further development of ingested parasites in their midgut and hence onward transmission. The results of a first-in-human trial assessing the safety, pharmacokinetics (PK) and transmission-reducing activity (TRA) of TB31F in adult Dutch volunteers are described below. Four groups of five healthy, malaria-naive F/M subjects are administered a single IV dose of respectively 0.1, 1, 3 and 10 mg/kg TB31F and are monitored until day (D)84 post-administration. Solicited local, solicited systemic, unsolicited, and serious adverse events (SAEs) are recorded until D7, D28, D84 and end of study, respectively. Titres of circulating TB31F mAb are measured by ELISA. TRA is assessed by standard membrane feeding assays (SMFA) using laboratory-reared *Anopheles stephensi* mosquitoes, cultured *P. falciparum* gametocytes and subjects' serum. At the time of abstract submission, group 1 completed follow-up through D28. Administration in group 1 was safe and well tolerated, with no SAEs or grade 3 AEs or laboratory abnormalities. Administration to groups 2-4 is anticipated to recommence by September 2020 at the latest, (pandemic) circumstances permitting, and be completed by early November 2020. We anticipate collection of AE data for all groups, and PK and TRA data for at least groups 1-3, to be completed up to ≥D7 in time for presentation at ASTMH 2020. Assuming TB31F is safe and well tolerated in the higher dose groups, we predict that administration of TB31F may provide transmission-blocking activity throughout an entire malaria transmission season.

679

EFFECT ON GAMETOCYTES OF THE FIXED-DOSE COMBINATION KAF156 (GANAPLACIDE) AND LUMEFANTRINE-SDF IN A PHASE 2 CLINICAL TRIAL

Cornelis Winnips, Celine Risterucci, Victoria Beindl

Novartis Pharma AG, Basel, Switzerland

In the lifecycle of a malaria parasite, maturation from the asexual stage into the sexual form (gametocytogenesis) is essential for the transmission from an infected human host to a mosquito vector. Gametocytes are thus a good target to break the circle of transmission. Only few antimalarial drugs act directly (primaquine and tafenoquine) or indirectly (artemisinine combination therapies (ACTs)) on gametocytes. A novel antimalarial agent KAF156 (ganaplacide) in combination with Lumefantrine-Solid Dispersion Formulation (LUM-SDF) is currently in clinical development for the treatment of uncomplicated *Plasmodium falciparum* malaria in adults and children. KAF156 already demonstrated *in vitro* gametocyte maturation and transmission-blocking activity. Herein, we investigated the potential gametocyte reducing activity of KAF156/LUM-SDF *in vivo*. 322 patients in Africa and Asia were treated with different dose combinations of KAF156 (200 mg, 400 mg, 800 mg) and LUM-SDF (480 mg, 960 mg) for 1, 2 or 3 days. Upon blood sampling before and after treatment and microscopic examination of each sample on Giemsa stained thick films, gametocytes were detected in 9 out of 322 patients (3%) at baseline. Gametocytes were cleared in 2 out of 9 patients at Day 0 (on the day patients were treated), in 3 out of 9 patients at Day 1, in 5 out of 9 patients at Day 2, in 7 out of 9 patients at Day 3 and in all patients at Day 4. A median gametocyte clearance time of 48 h was observed. Using linear regression modelling, KAF156/LUM-SDF decreased gametocyte counts by 26.3% per 24 h. Dose dependency of gametocyte clearance could not be observed due to the low number of patients. These data suggest that the superior *in-vitro* gametocidal activity of KAF156 also occurs *in-vivo*. Overall, this drug combination shows promising transmission-blocking potential in malaria patients.

680

THE EFFECT OF DIHYDROARTEMISININ-PIPERAQUINE INTERMITTENT PREVENTIVE TREATMENT DURING PREGNANCY COMPARED TO SULFADOXINE-PYRIMETHAMINE ON CLINICAL MALARIA AND *PLASMODIUM FALCIPARUM* INFECTION DURING INFANCY

Liana R. Andronescu[1], Yuanyuan Liang[1], Martin Kachingwe[2], Andy Bauleni[2], Witness Kachepa[2], Julie R. Gutman[3], Jobiba Chinkhumba[2], Don P. Mathanga[2], Miriam K. Laufer[1]

[1]*University of Maryland Baltimore, Baltimore, MD, United States,* [2]*Malaria Alert Center, University of Malawi College of Medicine, Blantyre, Malawi,* [3]*Centers for Disease Control and Prevention, Malaria Branch, Atlanta, GA, United States*

Due to increasing parasite resistance to sulfadoxine-pyrimethamine (SP), intermittent preventive treatment of malaria during pregnancy (IPTp) with dihydroartemisinin-piperaquine (DP) provides greater protection from malaria in pregnancy than the standard of care, IPTp-SP. Infants born to mothers enrolled in a trial of pregnant women randomized to receive either monthly SP or DP in Malawi were followed for up to 24 months after birth to assess whether prenatal malaria exposure affects risk of infant infection. Infants were seen every 3 months for routine visits and in between for acute illness. Clinical malaria was diagnosed and treated at any visit where patients presented with fever and a positive rapid diagnostic test or positive blood smear. Of 387 infants in the study, 192 infants were born to mothers randomized to SP and 195 to mothers receiving DP. There was no significant difference in the number of incident clinical malaria episodes based on IPTp exposure (0.25 (SP) vs. 0.27 (DP) episodes per person-year (PPY), incidence rate ratio (IRR): 1.17, 95% CI: 0.78 - 1.75, p=0.45). Males exposed to DP had twice the incident rate for clinical malaria compared to those exposed to SP (IRR: 2.03, 95% CI: 1.08-3.82, p=0.03). Among infants exposed to DP, there was a 23% higher rate of incident *Plasmodium falciparum (Pf)* infections compared to SP (0.81 (SP) vs. 0.98 (DP) infections PPY, IRR: 1.23, 95% CI: 0.99 - 1.53, p=0.06). Participants over 12 months of age exposed to DP had two times the incident infections compared to those exposed to SP (IRR: 2.14 95% CI: 1.17-3.93, p=0.01). There was no significant difference in time-to-first episode of clinical malaria (hazard ratio (HR) 0.93, 95% CI: 0.58-1.50, p=0.78) or time-to-first *Pf* infection (median survival time 299 days (SP) vs. 272 (DP), HR: 1.06, 95% CI: 0.80-1.40, p=0.68) based on IPTp exposure. Malaria incidence was lower than expected. The analysis has limited power to detect small differences, but evidence suggests DP may be associated with higher rates of clinical malaria and *Pf* infection among exposed infants compared to SP.

681

OPEN-LABEL RANDOMIZED CONTROLLED TRIAL OF ARTEMETHER-LUMEFANTRINE ANTIMALARIAL CHEMOPROPHYLAXIS FOR FOREST GOERS IN CAMBODIA

Rupam Tripura[1], Tom Peto[1], James Callery[1], Chhoeun Heng[1], Mom Ean[1], Lorenz von Seidlein[1], Franca Conradis-Jansen[1], Monnaphat Jongdeepaisal[1], Panarasri Khonputsa[1], Chris Pell[1], Worarat Khuenpetch[1], Siv Sovannaroth[2], **Richard J. Maude**[1]

[1]*Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand,* [2]*National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia*

In the Greater Mekong Subregion (GMS) adults are at highest risk for malaria. The most relevant disease vectors bite during daytime and outdoors which makes forest work a high-risk activity for malaria. The absence of effective vector control strategies and limited periods of exposure during forest visits, plus the need for some to travel far to attend a health facility, suggest that chemoprophylaxis could be an appropriate strategy to protect forest workers against malaria. We undertook an *in vivo* clinical assessment of prophylaxis to prevent malaria in 4400 participant episodes in villages in Stung Treng Province, Cambodia. Eligible subjects were adult forest goers planning to visit the forest within 72 hours and stay overnight. The subjects were randomized in a one-to-one ratio between artemether-lumefantrine (AL) ACT and a multivitamin preparation with no antimalarial activity. Efficacy of AL was assessed through follow up visits 28 days (+/-7 days) after returning from the forest when temperature, symptom questionnaires, brief physical examinations, and malaria parasite PCR, and, in selected individuals, parasite genetics were performed. Episodes of confirmed clinical malaria among study participants at any time point between enrolment and follow-up were also recorded. Co-primary endpoints were 28-day PCR positivity rate of *Plasmodium* infections of any species and the proportion of participants with confirmed clinical malaria of any species reported between day 0 and day 28. Subgroups of participants carried GPS loggers and were interviewed about their travel and activities in the forest and use of different preventive measures. In-depth interviews of forest goers, community healthcare workers and policymakers were conducted to assess the feasibility of prophylaxis as an intervention in Cambodia, Lao PDR and Thailand.

682

MALARIA DURING PREGNANCY: EFFECTS OF IPTP WITH DP, DPAZ AND SP ON MATERNAL IMMUNE ACTIVATION AND *PLASMODIUM FALCIPARUM* CLEARANCE

Amaya Ortega[1], Feiko ter Kuile[2], Mwayi Madanitsa[3], Stephen Rogerson[1]

[1]*The Peter Doherty Institute for Infection and Immunity, Melbourne, Australia,* [2]*Centre for Global Health Research, Kisumu, Kenya,* [3]*Department of Community Health University of Malawi, Blantyre, Malawi*

IPTp (Intermittent preventive treatment) is an important component of malaria prevention in pregnant women in malaria endemic areas. But emergence of resistance to sulphadoxine-pyrimethamine (SP), which is recommended by WHO, is compromising the efficacy of IPTp in countries like Malawi, and alternative agents are needed. One such, DP (dihydroartemisinin-piperaquine), has entered human clinical trials. The (Improving PRegnancy Outcomes with intermittent PreVEntive treatment in Africa) IMPROVE trial (NCT03208179), has explored the feasibility of replacing SP with DP for IPTp, alone or combined with the antibiotic azithromycin. Our lab is currently exploring *in vitro* the effect of the different IPTp drugs (SP, DP and AZ) on the maternal immune response. We are measuring cytokine responses to *Plasmodium falciparum* infected red blood cells (iRBCs) and other stimuli, and ability of maternal immune cells to take up iRBCs. To do that, we are using PBMCs collected at XX, XX and XX from Malawian women enrolled in the IMPROVE trial (who received different IPTp regimes during their pregnancy). We will examine paired cytokine responses and PBMCs activity using samples collected from the same patient before and after receiving IPTp, and/or at study enrolment and at delivery, using parametric and non-parametric tests as appropriate. Additionally, we are using PBMCs from Australian donors who have never been exposed to *P. falciparum* to examine whether *in vitro* exposure to these drugs alters their immune responses. We hypothesize that AZ will decrease cytokine secretion and that compared to SP, DP will be superior at inducing monocyte clearance of *P. falciparum* iRBCs.

683

A PHASE 2, MULTI-CENTER, RANDOMIZED, ACTIVE CONTROL (COARTEM), OPEN-LABEL, DOSE-ESCALATION STUDY TO DETERMINE SAFETY OF SINGLE (QD) AND MULTIPLE (3 QD) DOSES OF CIPARGAMIN (KAE609), GIVEN TO ADULTS WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

Preetam Gandhi[1], Katalin Csermak Renner[1], Esther Schmitt[1], Guoqin Su[2], Vinay Kumar Venishetty[3], Sarita Barsainya[4]

[1]*Novartis Pharma AG, Basel, Switzerland,* [2]*Novartis Pharma, East Hanover, NJ, United States,* [3]*Novartis Pharma, Hyderabad, India,* [4]*Novartis Pharma, Hyderbad, India*

KAE609 is a PfATP4 inhibitor with potent and fast-acting schizonticidal activity. Hepatic safety signal (transient asymptomatic grade 2-3 Liver Function Test elevation) was identified in two studies in the early

development program. KAE609A2202 was designed to evaluate hepatic safety of KAE609 at single and multiple ascending doses in adults with *Plasmodium falciparum* malaria (500-50000 parasites/µl). This study evaluated doses ranging from 10mg SD to 150 mg SD and 10 mg QDx3 days to 50mg QD x 3 days in 5 sequential cohorts. The primary safety variable was the occurrence of at least two CTCAE grades increase from baseline in ALT or AST during the 4 weeks study period. Efficacy (initial parasite clearance and 29 day cure rate) were evaluated as secondary variables. A total of 188 (KAE609: 137 and Coartem: 51) patients were randomized in five cohorts. Subject demographics and baseline characteristics were comparable across treatment groups except higher baseline parasite density in cohorts 4 and 5. Overall, 2/135 (1.48%) patients treated with KAE609 had at least two CTCAE grades increase from baseline in ALT or AST compared to 2/51 (3.92%) patients treated with Coartem. Hepatic adverse events were reported in 10/135 (7.4%) patients treated with KAE609 compared to 6/51 (11.8%) patients treated with Coartem. KAE609 achieved PCR corrected ACPR at day 29 in the range of 68.2%-90% across treatment groups in comparison to 94.1% cure rate in pooled Coartem group. Parasite clearance time (PCT) was shorter in patients treated with KAE609 (mean: 8.0 to 27.7 hours) compared to those treated with Coartem (mean: 36.2 hours). Incidence of recrudescence and reinfection with KAE609 was 17.8% and 7.4% compared to 2% each with Coartem. No subject treated with KAE609 had early treatment failure. In conclusion, KAE609 in the range of 10 mg to 150 mg were generally safe and well tolerated in adult patients with uncomplicated malaria caused by *P. falciparum*. The cure rate observed with KAE609 was as expected with a monotherapy. KAE609 continues to provide evidence of fast parasite clearance (<12 hours) at doses ≥25 mg SD or QDx3 days.

684

DIGITIZING PAPER-BASED HEALTH FACILITY REGISTERS USING SCANFORM: A NOVEL APPROACH FOR IMPROVING THE QUALITY, TIMELINESS, AND USE OF ROUTINE SURVEILLANCE DATA

Aaron M. Samuels[1], Oliver Towett[2], William Wu[3], Brian Seda[1], Jiehua Chen[3], Kennedy Oruenjo[4], Simon K. Kariuki[2], Feiko O. ter Kuile[5]

[1]*Centers for Disease Control and Prevention, Kisumu, Kenya,* [2]*Kenya Medical Research Institute, Kisumu, Kenya,* [3]*Quantitative Engineering Design, Warsaw, Poland,* [4]*Siaya County Department of Health, Government of Kenya, Siaya, Kenya,* [5]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom*

In Kenya, patient-level health surveillance data are manually entered into paper-based registers at each health facility (MoH-405); these data are aggregated monthly by MoH staff, transferred to summary registers (MoH-711), and then finally entered into the District Health Information Software 2 (DHIS2). Each step may introduce human errors. To improve the quality and timeliness of surveillance data reported into DHIS2 we redesigned the MoH registers using ScanForm, a technology that digitizes handwritten data using photographs of the registry pages taken with an Android-based phone application. To limit misinterpretation of free text, ScanForm registers utilize check boxes with standardized responses where possible. Encrypted images of the de-identified register pages are uploaded onto secure cloud-based servers where data are recognized by deep convolutional neural networks. High accuracy of character identification (>99%) and summary data are achieved through machine learning and automated generation of summary tables, respectively. From September 2018 to January 2020 study staff in 8 health-facilities in western Kenya prospectively transcribed data from MoH-405 registers into ScanForm registers, from which the automated summary data were used as the gold standard. Fifty-three MoH-405 variables were collected, of which 7 were reported into the MoH-711 and then DHIS2. A total of 1088 aggregated data summaries were collected in the MoH-405; all were reported in DHIS2. Overall concordance of the 1088 summary data elements between ScanForm automated summaries and DHIS2 summary data was 43.2%. Concordance of between the MoH-711 and ScanForm summaries was 42.6%. MoH-711 summaries were 92.8% concordant with DHIS2 entries from the same clinics and time periods. Data reporting from MoH registers to DHIS2 was complete, but frequent errors in manual data aggregation and transcribing resulted in poor quality of reported data into the DHIS2. ScanForm has the potential to improve data quality through standardization of responses and automated summaries and allows for near real-time evaluation of individual-level data.

685

DEVELOPMENT OF A NEW CRYOVIAL WITH SEPTUM FOR USE WITH CRYOPRESERVED HUMAN EUKARYOTIC CELL VACCINES AND PRODUCTS COMPATIBLE WITH STORAGE BELOW -150°C

Eric Robert James[1], Benjamin Hunt[2], Henry Huang[1], Samantha Slavin[1], Oluwatobi Fagbohun[1], B Kim Lee Sim[1], Yonas Abebe[1], Anita K. Manoj[1], Robertt S. Abrams[1], Stephen L. Hoffman[1]

[1]*Sanaria, Rockville, MD, United States,* [2]*SiO2 Material Science, Auburn, AL, United States*

The live, attenuated, whole eukaryotic cell vaccine against malaria, Sanaria®PfSPZ Vaccine, and related products, must be cryopreserved and stored below -150 °C to maintain stability and meet regulatory requirements. Suitable containers that include a septum for syringe/needle access, that maintain sterility, are tamper evident, can withstand the rigors of cryopreservation, and can be used in any environment, are not commercially available; this represents a major bottleneck for many biological products. We have developed a novel cryovial with a snap-in cap comprised of polypropylene with a co-molded integral thermoplastic elastomer septum and a tamper evident seal. Our new cryovials have passed USP container closure integrity testing (CCIT) showing operational qualification and validation. The cryovials are also compatible with high throughput, high volume fill-finish vaccine manufacture and cryopreservation. The vials are labeled pre-sterilization and packaged in SBS-format 8 x 12 array racks with lids. Caps are packaged on cap carriers also in the SBS-format. During the fill-finish operation, the vials are robotically filled and capped with a semi-automated capper. Laminated foil seals (96 random access with tab) are heat annealed using a semi-automated sealer, to the vials' rims to enclose the septum beneath in a sterile compartment and provide and container closure integrity. The seals also provide tamper evidence. Cryopreservation is achieved using a controlled rate freezer with storage and shipping using standard liquid nitrogen vapor phase equipment. In the immunization clinic, the cryovial is thawed using an automated dry thawing device, and the foil seal is removed to access the septum. Diluent is added by needle/syringe through the septum, mixed, and diluted vaccine withdrawn for direct venous inoculation in a manner identical to standard vaccine vials. These new cryovials are now being used in the Phase 3 cGMP compliant manufacture of the aseptic, purified, cryopreserved, metabolically active, non-replicating, whole sporozoite PfSPZ Vaccine, and will be used in Phase 3 clinical trials beginning in 2021.

686

PHASE 2 DOUBLE-BLIND, FAMILY COMPOUND RANDOMIZED, COMPARATOR-CONTROLLED TRIAL OF PFS230D1M-EPA/AS01: VACCINE COMMUNITY TRIAL PROGRESS AND PILOT RESULTS

Issaka Sagara[1], Sara A. Healy[2], Jennifer Kwan[3], Abdoulaye Katile[1], Bourama Kamate[1], Mamady Kone[1], Jon Fintzi[4], Bruce Swihart[4], Mahamadou S. Sissoko[1], Amatigue Zeguime[1], Kelly Rausch[2], David L. Narum[2], Puthupparampil Scaria[5], Nicholas J. MacDonald[2], Daming Zhu[2], Daman Sylla[1], Mamadou Coulibaly[1], Jen C. Hume[2], Amagana Dolo[1], Sekou F. Traore[1], Patrick E. Duffy[2]

[1]*University of Bamako, Bamako, Mali,* [2]*Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, Rockville, MD, United States,* [3]*Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy and Infectious Diseases,*

Bethesda, MD, United States, [4]Biostatistical Research Branch, National Institute of Allergy and Infectious Diseases, Rockville, MD, United States, [5]2Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, Rockville, MD, United States

Transmission blocking vaccines prevent mosquito infections and community spread of malaria parasites, but require durable functional antibody responses and need to be safe to administer at the population level for elimination efforts. A phase 2 double-blind, 1-1 randomized/ family compound, comparator-controlled trial in Mali is underway to assess safety, tolerability, vaccine activity and vaccine efficacy of Pfs230D1M (40 ug) conjugated to ExoProtein A (EPA) and formulated with AS01 adjuvant versus comparators (Havrix, Typhim Vi, and Menactra) at a community level. The sample was drawn using a census of compounds in Doneguebougou, Mali and an adjacent village. Family compounds were aggregated by proximity and mosquito habitat into Vaccine Units (VU; n=137) and those VUs were randomly assigned to receive the TBV (Pfs230D1M-EPA/AS01, 40 µg) or comparators at a 0, 1, 2 month vaccination schedule in all eligible subjects 5 years of age or older. Unvaccinated children 1-4 years of age received AL treatment, prior to their VU receipt of dose #3, for parasitemia endpoints. All vaccinated subjects were treated with AL prior to dose #1 and children 5-8 years of age were treated with AL prior to dose #3. The trial began with an age de-escalation pilot phase in thirty healthy 9-18-year old children followed by thirty 5-8 year old children who received vaccine at 0,1 and ~4 months. No safety signals were observed in the blinded pilot study population, or the main phase of the study. In prior studies at this site, 9-18-year old children transmitted parasites most frequently to mosquitoes, and for this trial participated in mosquito direct skin feeding (DSF) assays as the primary endpoint of vaccine activity (reduction in the rate of positive DSF assays). The rate of positive blood smears in children 5-18 years of age is being followed as a measure of vaccine efficacy and secondary objective. Blinded results from the first year of study will be presented.

687

SPATIO-TEMPORAL TRENDS IN HOTSPOTS OF MALARIA DURING A MASS TEST AND TREAT TRIAL IN AN AREA OF HIGH TRANSMISSION IN WESTERN KENYA, 2013—2015

Monica Shah[1], Wycliffe Odongo[2], Lance A. Waller[3], Aaron M. Samuels[4], Norbert Odero[5], Kephas Otieno[6], Tony Sang[6], Kim A. Lindblade[4], Simon Kariuki[6], Meghna Desai[4], Michael R. Kramer[7]

[1]*Malaria Branch, Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention; Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA, United States, [2]Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya; Malaria Branch, Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Department of Biostatistics, Rollins School of Public Health, Emory University, Atlanta, GA, United States, [4]Malaria Branch, Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, GA, United States, [5]Duke Global Health Institute, Durham, NC, United States, [6]Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya, [7]Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA, United States*

The effectiveness of malaria hotspot-targeted interventions may depend on spatial and temporal stability. We described spatial and temporal trends in malaria hotspots in an area of high transmission of western Kenya over a two-year period and characterized trends for afebrile and febrile infections. As part a mass test and treat (MTaT) trial, >23,000 individuals were tested for malaria by rapid diagnostic test (RDT) during six rounds from 2013-2015 (October, January, and April). Spatial patterns of 1) RDT positive (irrespective of self-reported previous 48-hour fever history), and, among RDT positives, 2) afebrile and 3) febrile hotspots were analyzed via kernel smoothed density estimates. Stable hotspots were defined as areas with a significantly higher intensity of malaria cases across all six rounds compared to the population tested (Monte-Carlo permutation p-value < 0.025). The association between age distribution and hotspot stability was modeled with logistic regression. During MTaT rounds, RDT positivity ranged from 36-49%; half of RDT positives were afebrile. There were four stable hotspots of RDT positivity, five stable afebrile hotspots, and three stable febrile hotspots covering 16%, 21%, and 6% of the study area, respectively. Children <5 years had reduced odds of residing in stable RDT positive hotspots compared to participants ≥ 5 years (odds ratio [OR]: 0.89, 95% confidence interval [CI] 0.82—0.98, p=0.02). Age was not significantly associated with residing in stable afebrile (OR 0.94, 95% CI 0.86—1.03) or febrile (OR 0.92, 95% CI 0.78-1.07) hotspots. In an area of high transmission, hotspots of afebrile infection were more stable than hotspots of febrile infection. These findings are consistent with other studies which hypothesize that stable asymptomatic hotspots arise in areas of frequent malaria transmission, leading to greater population immunity and, therefore, reduced severe and symptomatic disease. Characterization of malaria hotspots with consideration of immunity-related factors such as age and clinical illness could inform the utility of spatially-targeted approaches.

688

FIRST MALARIA REPORT OF ASYMPTOMATIC CASES IN NATIVE COMMUNITIES OF CONDORCANQUI, PERU

Pershing Bustamante-Chauca[1], Miguel Bernal[2], Lizandro Gonzales[1], Rafael Tapia-Limonchi[3], Ananias A. Escalante[4], Juan R. Tejedo[3], **Stella M. Chenet**[3]

[1]*Dirección Regional de Salud (DIRESA)-Amazonas, Chachapoyas, Peru, [2]Red de Salud Condorcanqui, Chachapoyas, Peru, [3]UNTRM, Chachapoyas, Peru, [4]Temple University, Philadelphia, PA, United States*

Malaria remains a serious health threat in the Amazonas Department of Peru. Approximately 95% of the cases from Amazonas are found in the Río Santiago district (Condorcanqui Province), an area where 93 native communities live on the banks of Santiago River, with no electricity, drinking water, or road access, having the river as the primary means of transportation. From 705 *P. vivax* cases reported in 2018, the number of cases increased up to 1,843 in 2019, including 807 new *P. falciparum* cases. It is important to mention that since 2015 only four *P. falciparum* cases (most likely imported) were previously reported in this area. As part of malaria surveillance by *DIRESA-Amazonas* and *Red de Salud Condorcanqui*, around 47.4% (2718) of the people in 21 communities were screened for malaria infections between January 31st and February 10th, 2020 in Río Santiago. Although rapid diagnostic tests (RDTs) were used in symptomatic patients, microscopy confirmation was also performed. In summary, 18 out of the 21 communities included in the study, reported positive cases, from which Nueva Esperanza and Alianza Progreso were the most affected. According to the results, malaria prevalence in the population of Condorcanqui was 5.4%. Thus, 60% (131/220) of the cases were asymptomatic at the moment of collecting the sample while 40% (89/220) were symptomatic. *Plasmodium falciparum* was found in 20.91% and *P. vivax* in 76.36% of the cases; 2.73% corresponded to mixed infections. The odds ratio of asymptomatic cases in *P. falciparum* (odds ratio [OR] = 3.32; 95% confidence interval [CI] = 1.51-7.3) was greater than in *P. vivax* (odds ratio [OR] = 0.3; 95% confidence interval [CI] = 0.14-0.66). Most of the 220 confirmed malaria cases were children under the age of eleven (46.36%). This is the first report of asymptomatic malaria cases and autochthonous *P. falciparum* cases in Condorcanqui. Asymptomatic cases pose a challenge to control efforts since they do not seek treatment, and therefore could act as malaria reservoirs. Timely identification and treatment, particularly of asymptomatic cases, are critical to achieve malaria control and possible elimination in this area.

690

PLASMODIUM FALCIPARUM AND *P. MALARIAE* INFECTION AMONG SYMPTOMATIC PATIENTS PRESENTING TO AN URBAN EMERGENCY DEPARTMENT IN DOUALA, CAMEROON

Yannick M. Etoundi[1], Daniel Z. Hodson[2], Glwadys Cheteug N[3], Tatiana Nganso[4], Justin Goodwin[2], Martina Wade[5], Yap Boum II[6], Carole E. Eboumbou Moukoko[4], Sunil Parikh[5]

[1]*Douala Military Hospital, Douala, Cameroon,* [2]*Yale School of Medicine, New Haven, CT, United States,* [3]*University of Buea, Buea, Cameroon,* [4]*Pasteur Center of Cameroun, Yaoundé, Cameroon,* [5]*Yale School of Public Health, New Haven, CT, United States,* [6]*Médecins Sans Frontières/ Epicentre, Yaoundé, Cameroon*

Recent molecular-based diagnostics across sub-Saharan Africa have uncovered a surprisingly high prevalence of *Plasmodium malariae* (Pm), a species classically associated with chronic, low level parasitemia. Cameroon is highly malaria endemic; however, data on Pm incidence outside of Yaoundé, the capital, are lacking. Between June and November 2018, 554 febrile patients ≥6 months old were recruited at the time of presentation to the district-level Military Hospital Emergency Department in Douala. From venous blood, pan-species rapid diagnostic test (RDT), blood smears, dried blood spots (DBS), and pellet samples were taken. Speciation was conducted on DBS and RBC pellets using distinct PCR protocols, with RBC pellet-based samples as the gold standard. The median age of participants was 28 years old (IQR 19-45); half the patients were female. By RDT, 66/548 (12%) were HRP-2 positive (Pf only), 68/548 (12%) were HRP-2 and pLDH positive (Pf or mixed), and 2/548 (0.4%) were pLDH-only (non-Pf species). Initial microscopy read found 85/554 (15%) Pf, 1/554 Pm, and 1/554 *P. ovale* mono-infection (2nd read pending). Geometric mean parasitemia was 9648/µL. PCR from DBS yielded 165/547 (30%) Pf-positive, including 4 Pf/Pm co-infections. PCR from RBC pellets yielded 209/528 (40%) Pf mono-infections, 15/528 (3%) Pm mono-infections, and 9/528 (2%) Pf/Pm co-infection; an overall higher rate of positivity than DBS samples. Pf burden appeared to peak in August, while Pm positivity was highest in September. Compared to PCR from RBC pellet, RDT had an overall sensitivity of 54% and specificity of 98%. Of the Pm cases, RDT detected 1/15 (7%) mono-infections and 4/9 (44%) Pf/Pm co-infections. For Pf, microscopy had a sensitivity of 38% and specificity of 99%. PCR from DBS had a sensitivity of 63% and specificity of 93%, but identified none of the Pm mono-infections and 1/9 (11%) of the Pf/Pm co-infections. Even among symptomatic patients in a clinical setting, *P. malariae* is poorly detected by RDT and microscopy. Future research should directly compare different DNA extraction and sample types, as well as PCR protocols for detecting Pm and other less studied species.

691

HIDDEN RESERVOIR: ASYMPTOMATIC *PLASMODIUM FALCIPARUM* PREVALENCE IN MALAWIAN ADOLESCENTS AND ADULTS, 2015-2016

Hillary M. Topazian[1], Austin Gumbo[2], Sydney Puerto-Meredith[1], Ruth Njiko[3], Michael Kayange[2], David Mwalilino[4], Bernard Mvula[4], Gerald Tegha[3], Tisungane Mvalo[3], Steven R. Meshnick[1], Irving Hoffman[1], Jonathan J. Juliano[1]

[1]*University of North Carolina at Chapel Hill, Chapel Hill, NC, United States,* [2]*National Malaria Control Programme, Malawi Ministry of Health, Lilongwe, Malawi,* [3]*University of North Carolina Project-Malawi, Lilongwe, Malawi,* [4]*National HIV Reference Laboratory, Malawi Ministry of Health, Lilongwe, Malawi*

Malaria remains an important cause of childhood morbidity and mortality in Malawi, with an estimated *Plasmodium falciparum* rate of 18-19% in children 2-10 years in 2015-2016. While children report the highest rates of clinical disease, adults are thought to be an important reservoir to sustained transmission due to persistent asymptomatic infection. However, little is known about the prevalence of adult asymptomatic malaria infections in Malawi. The Malawi Demographic and Health Survey V was a nationally representative household survey which collected dried blood spots from 14,780 asymptomatic individuals ages 15-54 between October 2015 and February 2016. In order to understand the asymptomatic burden of malaria in Malawi, we performed quantitative PCR on 7,393 samples to detect parasitemia. After incorporating survey weights, 2170 were positive for *P. falciparum*, resulting in an overall prevalence of 31.1%. The majority of positive samples (1207/2170, 55.6%) had parasitemias ≤10 parasites/µL. Prevalence estimates were higher in the Central (914/2567, 35.6; p<0.001) and Southern (1001/3326, 30.1; p=0.02) regions compared to the North (254/1076, 23.6). Parasite prevalence was higher in rural (2018/5876, 34.3; p<0.001) versus urban areas (152/1093, 13.9). Adolescents and young adults ages 15-24 had a prevalence of 36.1 (1096/3031); as age increased, malaria prevalence declined to 24.5 among those 45-54 years (142/581, p<0.001). Prevalence estimates were highest among individuals in the poorest wealth quintile (530/1242, 42.7), and lowest among those in the wealthiest quintile (260/1551, 16.7; p<0.001). Individuals sleeping under a bednet treated with alpha-cypermethrin or deltamethrin had a slightly lower prevalence (254/897, 28.4) than those sleeping under a permethrin treated net (507/1649, 30.8; p=0.4). Our findings demonstrate a higher parasite prevalence in adults than published contemporary estimates among children. Understanding the prevalence and spatial distribution of underlying infection is essential for implementation and evaluation of future interventions.

692

HIGHER ODDS OF SYMPTOMATIC *PLASMODIUM FALCIPARUM* INFECTION WHEN EXPOSED TO NOVEL COMPARED TO RECURRENT MALARIA INFECTIONS OVER TIME

Kelsey M. Sumner[1], Elizabeth Freedman[2], Lucy Abel[3], Andrew Obala[4], Steven R. Meshnick[1], Brian W. Pence[1], Wendy Prudhomme-O'Meara[2], Steve M. Taylor[2]

[1]*University of North Carolina at Chapel Hill, Chapel Hill, NC, United States,* [2]*Duke University, Durham, NC, United States,* [3]*Moi Teaching and Referral Hospital, Eldoret, Kenya,* [4]*Moi University, Eldoret, Kenya*

Repeated exposure to malaria infections could protect against symptomatic progression, as people develop partial immunity to infections acquired over time. We investigated how recurrent *Plasmodium falciparum* infections affected the odds of developing symptomatic compared to asymptomatic malaria infections. Using a 14-month longitudinal cohort in Webuye, Kenya, we used amplicon deep sequencing of two polymorphic genes (*pfama1* and *pfcsp*) to assess overlap of parasite genotypes (represented by haplotypes) acquired within an individual's successive infections. We hypothesized that infections with novel haplotypes would be associated with an increased odds of symptomatic malaria. After censoring initial infections, we observed 534 asymptomatic and 88 symptomatic infections across 239 participants. We observed 139 *pfcsp* haplotypes, and each infection was classified as harboring recurrent or novel haplotypes for that individual. Comparing the odds of symptomatic malaria across infections with all novel haplotypes (N=169) versus any recurrent haplotypes (N=453), a multi-level logistic regression model was run controlling for within-individual random effects, time, and age. Model results found that infections with all novel haplotypes had a higher odds of symptomatic malaria [OR: 1.99, 95% CI: 1.18 to 3.35]. To test if age modified the haplotype classifications and symptomatic disease progression, we re-ran the model stratified by age category (<5 years, 5-15 years, >15 years). Model results found that children <5 years [OR: 6.04, 95% CI: 0.77 to 47.69] had a higher odds of symptomatic infection compared to participants 5-15 years [OR: 1.59, 95% CI: 0.78 to 3.24] and >15 years [OR: 1.93, 95% CI: 0.64 to 5.77], but results were not statistically significant. Results were similar but not statistically significant for *pfama1*. These results confirm that infections with all novel haplotypes compared to infections with any recurrent haplotypes had an increased odds of symptomatic malaria. Our results are consistent with a model in which disease-limiting immunity is acquired by successive infections in highly-endemic settings.

693

RECENT MOLECULAR ASSESSMENT OF *PLASMODIUM VIVAX* AND *P. FALCIPARUM* ASYMPTOMATIC AND SYMPTOMATIC INFECTIONS IN BOTSWANA

Isaac K. Quaye

Regent University of College of Science and Technology, Accra, Ghana

In 2016 we reported for the first time on the presence of *Plasmodium vivax (Pv)* in Botswana through an active cross-sectional study. In 2018, we carried out another active survey across 10 districts to assess the changes if any, in the prevalence of *Pv* and other human *Plasmodium* species in the country. A total of 1615 children between 2-<12 years old, were assessed for *Plasmodium* species. The mean age of all the subjects was 5.40 years, (interquartile range 2-13). The number of subjects <5 years was 81.7% =>5 years formed 18.3%. Symptomatic subjects who were from the clinics were 10.6%, with female gender constituting 49.9%. None of the participants had traveled outside the country for the past year. The *Plasmodium* species infections were as follows: *Pv*, 12.7%, *P. falciparum* (*Pf*), 12.5%, *Plasmodium ovale* (*Po*), 0.7%, *Plasmodium malariae* (*Pm*), 0.7%. The mixed infections were (*Pf* and *Pv*), 2.4%; (Pf and Po), 0.6% (Pv and Po), 0%; (*Pv* and *Pm*), 0.1%; (*Pf* and *Pm*), 0.7%. The infections were largely asymptomatic. There was 8.8% variation of risk with residence and *Pv* infection. Binary logistic regression showed that the odds of being infected with *Pv* was highest in South East and Kweneng East: OR 2.19,95%CI(1.02-4.74); and OR 2.43, 95%CI(1.18-5.03). *Pv* appears to be expanding within the asymptomatic population in Botswana We affirm that *Pv* is still significantly present in Botswana, which requires the attention of the National Malaria Control Program (NMCP) regarding intervention and mitigation of the spread in addition to making it a key focus in the elimination agenda.

694

ASSESSMENT OF PVMSP8 AS SEROLOGICAL MARKER OF RECENT *PLASMODIUM VIVAX* EXPOSURE IN THE PERUVIAN AMAZON

Katherine Garro*[1], Elizabeth Villasis*[1], Angel Rosas-Aguirre*[2], Pamela Rodriguez[1], Jason Rosado[3], Anthony Gave[1], Mitchel Guzman[1], Paulo Manrique[1], Niko Speybroeck[4], Joseph Vinetz[5], Dionicia Gamboa[6], Katherine Torres[6]

[1]*Laboratorio ICEMR-Amazonia, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía Abraham Vaisberg Wolach, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru,* [2]*Fund for Scientific Research FNRS, Brussels, Belgium,* [3]*Unit of Malaria, Parasites and hosts, Institut Pasteur, Paris, France,* [4]*Research Institute of Health and Society (IRSS). Université catholique de Louvain, Brussels, Belgium,* [5]*Section of Infectious Diseases, Department of Internal Medicine, Yale School of Medicine, New Haven, CT, United States,* [6]*Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru*

Serological markers of recent malaria exposure can be used to guide malaria control efforts. After the production of *Plasmodium vivax* (Pv) merozoite surface protein 8 (PvMSP8) in our laboratory using a baculovirus expression system, we conducted a two-step validation process to assess IgG responses against this protein as potential malaria seromarker of Pv exposure in the Peruvian Amazon. In a first study, Luminex assays analyzed 422 plasma samples collected in January 2013 from two Amazonian population cohorts with known history of Pv exposure (monthly follow-up by PCR and/or microscopy). Although its slightly lower performance compared to anti-PvMSP10 (area under the curve AUC=0.78 [95%CI: 0.72-0.83]), antibodies to PvMSP8 were able to discriminate recent Pv exposure within the past five months (AUC=0.72 [95%CI: 0.67-0.78]). The sensitivity of dichotomized results (mean plus three SD of negative controls as cut-off) was the highest with confirmed infections occurring 7-30 days before sample collection (sensitivity 78.6 %). In a second study, ELISA assays analyzed all collected plasma samples during a cross-sectional population-based survey in October 2018 in eight malaria endemic communities from Mazan, Loreto. A first definition of Pv exposure was a positive Pv parasitological result (by microscopy and/or PCR) at the time of the sample collection, while a second definition included a positive Pv parasitological result and/or the antecedent of Pv malaria in the past month. Among the total 1250 participants, 81 (6.5%) had a Pv confirmed diagnosis, and 111 (8.9%) had a Pv confirmed diagnosis and/or history of Pv in the past month. IgG responses against PvMSP8 had good performance for identifying Pv exposure (AUC=0.76 [95%CI: 0.70-0.81]) using the first definition; (AUC=0.79 [95%CI: 0.74-0.83] using the second definition). The analysis by subgroups showed that the performance in identifying Pv exposure is greater in individuals ≥15 years (0.84 [95%CI: 0.77-0.91]) than in younger individuals (0.71 [95%CI: 0.75-0.78]). PvMSP8 seems to be a good marker of recent Pv exposure in low-moderate transmission settings of the Peruvian Amazon.

695

TRENDS IN MALARIA INDICATORS FROM DEMOGRAPHIC AND HEALTH SURVEYS IN SENEGAL FROM 2005 TO 2017

Malick Anne[1], Mamoudou Bocoum[1], Awa Niang[1], Ibrahima Diallo[1], Khady Camara[1], Adja Ndiaye Sall[1], Mayassine Diongue[2]

[1]*Senegal Health Ministry and Social Action, Podor, Senegal,* [2]*Public Health Cheickh Anta Diop University, Dakar, Senegal*

In 2017, according to the World Health Organization, the number of estimated malaria cases in the world was 219 million against 239 million in 2010 and 217 million in 2016. In Senegal, the fight against malaria has known Significant advances thus the proportional malaria morbidity went from 35% in 2001 to 3% in 2016. The proportional mortality went from 29% in 2001 to 2% in 2016. The country has recorded significant progress in terms of access to and use of Long-Lasting Insecticide-treated Mosquito Nets (LLINs). A gain of more than 20 points was made on access between 2010 and 2016.. Meanwhile, usage jumped 30 points between 2015 and 2016, while between 2010 and 2015, the gain was only 25 points. The study analyzed trends in malaria indicators in Senegal's demographic health surveys (DHS) from 2005 to 2017. It is a descriptive and analytical study of malaria indicators. The data come from EDS from 2005 to 2017. They were provided by the DHS program and were processed on STATA 15.0 and Excel. From a global perspective from 2005 to 2017, the main indicators of malaria experienced positive progress. However, between 2016 and 2017, a decrease was noted on almost all of these indicators. Ownership of nets has increased from 9% for long-acting treated nets (LLINs) in 2005 to 84% in 2017. The proportion of households with a net for two people who stayed last night has experienced an increase by type of mosquito net until 2016, from 3.1% in 2005 to 56.4% for ITNs, an increase of 53.3 percentage points. In 2017, these two indicators fell from 56.4% to 50.4% respectively for ITNs and from 55.4% to 50.3% for LLINs. The proportion of pregnant women living in rural areas who slept under LLINs the night before the survey is higher than in urban areas. Between 2010 and 2017, the indicator went from 33% to 67%, an increase of 103%. The results show that universal LLIN coverage campaigns have increased ownership and access, however there has been a gap between access and use. The fight against malaria has experienced significant progress and the analysis of the results obtained during the different DHS can allow the reorientation of the different control strategies.

696

TRENDS IN MALARIA MORBIDITY AND MORTALITY RATES IN UGANDA: A FOUR-YEAR RETROSPECTIVE STUDY

JohnBaptist Bwanika

Malaria Consortium, Kampala, Uganda

Approximately 40% of outpatient attendance and over 20% of hospital admissions in Uganda are reported as malaria. The US President Malaria Initiative's Malaria Action Program for Districts (MAPD) project supports the reduction of malaria morbidity and mortality through improving the implementation of core interventions, including service delivery

improvements through clinical and mortality audits, an approach not widely adopted in Uganda. This study compared severe morbidity and mortality in project and non-project districts. Health facility data collected through the health management information system between January 2016 and December 2019 were analyzed. Three outcomes were assessed: proportion of confirmed malaria cases classified as severe, proportion of malaria deaths in relation to malaria cases, and malaria mortality (malaria deaths per 100,000 population). 52 MAPD districts were pooled and compared to 84 pooled non-project districts, by year. A difference in difference (DID) estimator in mortality was computed to study the differential effect. The proportion of confirmed malaria cases classified as severe was similar (range: 3 - 5%) in both areas from 2016 to 2018. However, in 2019 - a period with frequent malaria upsurges especially in MAPD districts, this increased to 12% in the project area, but remained 5% in the comparator. The proportion of malaria-related deaths in the project area declined from 10% in 2016 to 4% in 2019, while in the comparator area it declined from 9% to 5%. Malaria mortality, however, decreased steadily from 16/100,000 in 2016 to 14 in 2017, to 7 in 2018, to 4 in 2019 in MAPD districts, whereas in the comparator area mortality dropped from 10 in 2016 to 4 in 2017, remained 4 in 2018, and then rose to 6 in 2019. This resulted in DID estimator for mortality of 2.35 (95%CI 2.24, 2.47). There were no statistically significant differences in data reporting rates between the two areas over time. This study suggests that MAPD districts were more likely to reduce malaria mortality than non-MAPD districts, despite malaria upsurges. Project interventions and implementation models may minimize mortality nationwide.

697

COMMUNITY PERCEPTIONS OF MALARIA RDT RESULTS IN A REGION OF HIGH MALARIA TRANSMISSION: WHAT INFLUENCES THEIR CONFIDENCE IN THE TEST RESULTS?

Judith Nekesa Mangeni[1], Josephine Malinga[2], Indrani Saran[3], Andrew Obala[1], Wendy O'Meara[2]

[1]*Moi University, Eldoret, Kenya,* [2]*Duke Global Health Institute, Durham, NC, United States,* [3]*Boston College, Newton, MA, United States*

WHO recommends test - based management of malaria infections. Perceptions of the community on malaria test results determine the actual success of such guidelines. We conducted a prospective cohort study embedded within an existing cohort of participants in three villages in Western Kenya. For this analysis, we examine a subset of participants who underwent RDT testing. Requests for RDT testing were initiated by the participants if they felt unwell. During the visit, participants were asked behavioral questions and perceptions towards malaria. Participants who were found positive on RDT were treated with AL. A total of 994 visits were made over a period of 30 months from June 2017 to November 2019 for a total of 224 participants. We recorded 410 (41.3%) episodes of malaria. Confidence in test results increases after RDT testing especially for those who test positive (X2=885.97, P < 0.001). There is a slight increase (14%) in confidence of the RDT test results with increasing number of RDTs done. Having a history of fever or viewing one's illness as severe affects their confidence in the test results (aOR; 2.2 [95%CI 1.5-3.2], aOR: 2.3 [95% CI 1.8-3.0). Community members are more likely to trust their RDT results as they get exposed to more tests especially if the test is positive. Unfortunately, people with fever or who view their illness as severe are less likely to trust a negative RDT test result. Successful efforts to reduce malaria in this region need to incorporate health education to correct misinformation and therefore ensure rational use of malaria treatment.

698

MULTI-STAKEHOLDER ENGAGEMENT FOR ACTIVE SURVEILLANCE OF MALARIA IN TWO TOWNSHIPS IN MYANMAR'S MANDALAY REGION

Poe Poe Aung[1], Nay Yi Yi Linn[2], Zaw Lin[2], Aung Thi[3], Tint Wai[4], Zaw Win Thein[1], Thura Htay[1], Zin Min Tun[1], Aye Kyawt Paing[1], Thant Khant Zaw[1], Christopher V Plowe[5], Myaing M Nyunt[5]

[1]*Duke Global Health Institute - Myanmar Program, Duke University, Yangon, Myanmar,* [2]*Vector-Borne Disease Control Program, Department of Public Health, Ministry of Health and Sports, Myanmar, Naypyitaw, Myanmar,* [3]*National Malaria Control Program, Department of Public Health, Ministry of Health and Sports, Myanmar, Naypyitaw, Myanmar,* [4]*Vector-Borne Disease Control Program, Department of Public Health, Ministry of Health and Sports, Myanmar, Mandalay, Myanmar,* [5]*Duke Global Health Institute, Duke University, Durham, NC, United States*

Multi-stakeholder engagement is essential for influencing organizational policies and reaching common goals such as effective malaria control and elimination. Access to the populations harboring transmissible malaria infection in remote border and conflict zones poses a challenge in many malaria-endemic nations, including Myanmar, which has set an elimination target of 2030. We piloted a multi-stakeholder engagement model in developing a sustainable active malaria surveillance program, designed to evaluate the distribution of asymptomatic malaria, using molecular surveillance, in 67 villages in two remote townships in Mandalay, one of six States and Regions slated for subnational elimination in Myanmar. The project was implemented in four phases. During Phase 1, with a goal to build political will and gain community buy-in and trust, a series of advocacy meetings and seminars were conducted with multiple layers of public health and political leadership, diverse village communities, social and religious leaders, and the national technical strategy group organized by the World Health Organization and consisting of governmental, national and international non-governmental, and ethnic health organizations involved in Myanmar's malaria elimination, as well as an independent expert committee. During Phase 2, the project was implemented with widespread participation from communities with a total combined population of 11,000, with the contribution of village malaria volunteers, led by the national malaria control program, and both Myanmar and U.S. research scientists of Myanmar. During Phase 3, study results were disseminated with all the stakeholders. Key findings and a policy brief were presented to the national programs and policy makers. During Phase 4, the Myanmar national malaria control program and public health leadership provided recommendations for next steps. This collaborative multi-stakeholder research model illustrates the potential for inclusive and sustainable long-term research in support of malaria elimination in Myanmar.

699

INCREASED GAMETOCYTE PRODUCTION AND MOSQUITO INFECTIVITY IN CHRONIC VERSUS INCIDENT *PLASMODIUM FALCIPARUM* INFECTIONS

William Stone[1], Aisata Barry[2], John Bradley[3], Moussa W. Guelbeogo[2], Kjerstin Lanke[4], Alphonse Ouedraogo[2], Issiaka Soulama[2], Issa N. Ouedraogo,[2], Samuel Serme[2], Lynn Grignard[1], Katie Patterson[1], Shehu S. Awandu[4], Mireille Ouedraogo[2], Casimire W. Tarama[2], Desiree S. Kargougou[2], Zongo Zoumanaba[2], Sodiomon B. Sirama[2], Matthias Marti[5], Chris Drakeley[1], Alfred B. Tiono[2], Teun Bousema[4]

[1]*LSHTM, London, United Kingdom,* [2]*Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso,* [3]*MRC Tropical Epidemiology Group, LSHTM, London, United Kingdom,* [4]*Radboud University Medical Center, Nijmegen, Netherlands,* [5]*University of Glasgow, Glasgow, United Kingdom*

In semi-immune individuals, higher *Plasmodium falciparum* asexual parasite densities are likely to result in higher gametocyte densities and greater transmission potential. However, it is unclear how the development

of symptoms interacts with gametocyte kinetics and infectivity in chronic and incident infections. Children aged 5-10 years were recruited from an area of high malaria transmission in Burkina Faso into two cohorts: an incident cohort in which new infections were followed after parasite clearance (n=48), and a chronic cohort in which asymptomatic infections were identified and followed (n=60). Parasite kinetics were assessed daily with repeated mosquito feeding assays to quantify transmission potential. 92% (44/48) of the incident cohort developed symptoms and were treated within 35 days, compared to 23% (14/60) of the chronic cohort. All but two individuals with chronic infection were gametocytaemic at enrollment, whereas only 35% (17/48) in the incident cohort developed gametocytes within 35 days. The abundance of *ap2-g* mRNA transcripts was positively associated with the observed conversion to gametocyte production (*i.e.* the ratio of gametocytes at day 14 to ring stage parasites at baseline) and was higher in chronic infections. Parasite multiplication rate was also positively associated with prospective gametocyte production. Most incident infections were cleared before gametocyte density was sufficiently high to infect mosquitoes. In contrast, chronic, asymptomatic infections represented a significant source of mosquito infections. If present, gametocytes were significantly less infectious if concurrent with malaria symptoms. Our observations support the notion that malaria transmission reduction may be expediated by enhanced case management, involving both symptom-screening and infection detection.

701

PLASMODIUM FALCIPARUM MALARIA PREVALENCE AND HEALTH SEEKING BEHAVIORS IN RURAL SUSSENDENGA DISTRICT, MOZAMBIQUE

Dominique Earland[1], Albino Bibe[2], Anísio Novela[3], João L. Ferrão[4], Kelly M. Searle[1]

[1]*University of Minnesota, Minneapolis, MN, United States,* [2]*Escola Secondária de Sussundenga, Sussundenga, Mozambique,* [3]*Direcção Distrital de Saúde de Sussundenga, Manica, Sussundenga, Mozambique,* [4]*Instituto Superior de Ciências de Educação, Beira, Mozambique*

The large-scale effectiveness of malaria control interventions is differential at international border settings with differing policies, such as that between Mozambique and Zimbabwe. Impacts of nationally directed malaria control interventions hinge on the understanding malaria transmission and prevention at the community level along international borders. Thus far, few studies have focused on central Mozambique. Our aim was to describe community level *P. falciparum* transmission dynamics and health seeking behaviors among residents of Sussundenga, Mozambique, a rural village bordering Zimbabwe in Manica Province with high malaria incidence reported at the Sussundenga-Sede health center (RHC). We conducted a cross-sectional community-based survey from December 2019 - February 2020. We used a random household sampling method, based on enumerated households from satellite imagery. All consenting participants completed a survey about malaria risk, prevention, and health seeking-behaviors, and received a *P. falciparum* malaria rapid diagnostic test (RDT). We enrolled 96 households with 358 individuals. The *P. falciparum* prevalence was 31.6% (95% CI [26.6-36.5]). Ninety-three percent of participants reported using the Sussundenge-Sede RHC for healthcare. Sixty-six percent of participants (N=233) experienced at least one malaria symptom in the past month, with self-reported fever most frequently reported (19.3%). Of these, 176 (76.5%) sought care in a health facility and 174 (79%) received an RDT with 130 (63%) positive. Of those with a positive RDT, 127 (97%) received Coartem®. Following treatment, 123 (97%) participants' symptoms resolved within a median of 3 days (IQR: 3-5) ranging from 2-14 days. In this high transmission setting, a high proportion of participants recognized malaria related symptoms then received a proper diagnostic test and treatment in a health facility . Future interventions that leverage this health seeking behavior and strengthen health systems for community interventions will improve malaria control and inform the efficacy of potential interventions at international borders.

702

PLASMODIUM FALCIPARUM MALARIA PREVALENCE AND HOUSEHOLD LEVEL RISK FACTORS IN RURAL MOZAMBIQUE ALONG THE ZIMBABWE BORDER

Kelly M. Searle[1], Dominique Earland[1], Albino Bibe[2], Anísio Novela[3], João L. Ferrão[4]

[1]*University of Minnesota, Minneapolis, MN, United States,* [2]*Escola Secondária de Sussundenga, Sussundenga, Mozambique,* [3]*Direcção Distrital de Saúde de Sussundenga, Manica, Sussundenga, Mozambique,* [4]*Instituto Superior de Ciências de Educação, Beira, Mozambique*

Malaria is one of the leading causes of morbidity and mortality in Mozambique with the 5th highest prevalence in the world and 3rd highest prevalence in Africa. Limited progress in control has been made over the past 20 years across Mozambique. The northern and central regions of the country have the highest *Plasmodium falciparum* incidence as reported through surveillance at government health facilities. Sussundenga Rural Municipality in Manica Province is one of most impacted areas in central Mozambique. Sussundenga is a rural village that lies along the Zimbabwe border, making evaluation of transmission and control policies integral for regional efforts. The objective of this analysis was to determine the household level risk factors for *P. falciparum* infection in Sussundenga to understand transmission dynamics and inform planning of control policies. We conducted a cross-sectional community-based survey from December 2019 - February 2020. We used a random household sampling method, based on enumerated households from satellite imagery. All consenting participants completed a survey about malaria risk, prevention, and health seeking-behaviors, and received a *P. falciparum* malaria rapid diagnostic test (RDT). We also conducted a household census and household survey to collect information on house construction, household movement and response to severe weather events, and recent visitors to the household. We enrolled 96 households with 358 individuals. The *P. falciparum* prevalence was 31.6% (95% CI [26.6-36.5]). We analyzed these data using a generalized estimating equations (GEE) logistic regression model to account for clustering of household variables. Our findings indicate local variation in household construction and economic status that are highly related to *P. falciparum* prevalence. This area has limited malaria control interventions and these findings can inform targeting of interventions based on the transmission dynamics within this community. These can be used to further enhance efforts toward regional malaria control and elimination efforts in this international border setting.

703

EXPLORING DISCREPANCIES BETWEEN MALARIA TEST POSITIVITY RATES FROM AUTOMATED READERS AND ROUTINE SURVEILLANCE DATA IN THE DEMOCRATIC REPUBLIC OF CONGO

Michael Humes[1], Radina Soebiyanto[1], Annē Linn[1], Lee Schroeder[2], Aboubacar Sadou[3], Erick Tshikamba[4], Eric Mukomena[5], Santiago Ferro[6]

[1]*President's Malaria Initiative, Washington, DC, United States,* [2]*Department of Pathology, University of Michigan School of Medicine, Ann Arbor, MI, United States,* [3]*President's Malaria Initiative, Kinshasa, Democratic Republic of the Congo,* [4]*Abt Associates, Lubumbashi, Democratic Republic of the Congo,* [5]*National Malaria Control Program, Kinshasa, Democratic Republic of the Congo,* [6]*Fio Corporation, Toronto, ON, Canada*

Test positivity rate (TPR) can be a strong predictor of malaria transmission and provides an estimate of temporal changes in malaria burden. TPR and malaria incidence can both display seasonal fluctuations, particularly in areas with unimodal rainfall patterns. TPR has a direct relationship with incidence but is comparatively less influenced by reporting rates or care seeking. TPR is important in assessing true burden of malaria, but its reporting is vulnerable to the pressure of provider non-adherence to test results, which warrants exploration. From early 2016 to mid-2019 automated malaria rapid diagnostic test (RDT) readers, Deki Readers, were deployed in health facilities across Haut Katanga and Lualaba, two

provinces in southern Democratic Republic of Congo (DRC) with a well-defined unimodal rainy season that runs from October to April. Previous research has shown that Deki Readers are as accurate at interpreting RDTs as trained healthcare workers. A retrospective review of malaria data was conducted, comparing TPRs reported from Deki Readers against TPRs reported on the DRC's Health Management Information System (HMIS), in the same facilities and same time period. Data from 102 health facilities that regularly used the Deki Readers in 2017 and 2018 were included. Paired t-test showed a statistically significant ($p<0.001$) difference in TPRs of 30.2 percentage points by source, with an average annual TPR from the Deki Readers of 23.6% (CI: 22.7 – 24.6), compared to 53.8% (CI: 52.6 – 55.0) from HMIS. Considerable variation of Deki Reader TPR, but not HMIS TPR, was found between seasons and tracks rainfall trends. The difference was more extreme during the low malaria transmission season (May – September), when Deki Readers reported a TPR of 16.3% (CI: 15.1 – 17.5) compared to 51.1% from HMIS (CI: 49.1 – 53.1). For the two-year period these data show the TPR from HMIS is 128% higher than the TPR from Deki Readers. Such a difference has considerable implications for true measures of cases, incidence and treatments. Automated RDT readers provide a unique opportunity to measure the pervasiveness and magnitude of potential HMIS reporting biases.

704

UNDERSTANDING MALARIA DISTRIBUTION AND LANDSCAPE USE IN TANZANIA: A MOLECULAR EPIDEMIOLOGY STUDY OF SCHOOL-AGED CHILDREN

Cedar Mitchell[1], Billy Ngasala[2], Frank Chacky[3], Ally Mohamed[3], Lwidiko Mhamilawa[2], Fabrizio Molteni[4], Ssanyu Nyinondi[5], Bilali Kabula[5], Erik Reaves[6], Naomi Serbantez[6], Chonge Kitojo[6], Twilumba Makene[2], Thwai Kyaw[1], Jonathan Parr[1], Michael Emch[1], Steven Meshnick[1], Jonathan Juliano[1]

[1]*University of North Carolina at Chapel Hill, Chapel Hill, NC, United States,* [2]*Muhimbili University of Health and Allied Sciences, Dar es Salaam, United Republic of Tanzania,* [3]*National Malaria Control Programme, Dodoma, United Republic of Tanzania,* [4]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [5]*RTI International, Dar es Salaam, United Republic of Tanzania,* [6]*US President's Malaria Initiative, Dar es Salaam, United Republic of Tanzania*

Malaria transmission in Tanzania is spatially heterogenous. Varying levels of transmission intensity throughout the country have been largely attributed to differing ecological patterns. However, the effects of human modifications of the local environment for agriculture or other land use activities have not been well defined across the diverse transmission settings in the country. Understanding the impact of human-modified land cover may help explain higher and lower areas of malaria prevalence. We evaluated the relationship between local environmental characteristics and malaria prevalence across low endemic to hyper-meso endemic malaria transmission intensity settings. We used 20,284 dried blood spots collected during a survey of 65,935 school children administrated in 2017 by the National Malaria Control Program. The samples were collected from asymptomatic individuals ages 5-16 years enrolled at 199 schools across 8 regions. *Plasmodium falciparum* parasitemia was measured initially using rapid diagnostic tests and later assessed by real-time PCR. Rapid diagnostic test results indicate an overall malaria prevalence of 18.5% across all sampled schools (range: 0-100% prevalence). Initial results from 15,498 of the 20,284 dried blood spots indicate an overall PCR prevalence of 20.5% across all schools (range: 0-85% prevalence). Environmental factors were derived from earth observation data and included: precipitation, land surface temperature, elevation, vegetation abundance and richness, and land use for irrigated and rainfed crops. We are linking malaria prevalence data and environmental predictors using GPS coordinates to determine how variations in the local environment and land cover influence malaria prevalence at the school, district, and regional levels. Understanding the impact of environmental processes and land use on malaria transmission intensity is increasingly important in settings like Tanzania. These results can be used to inform targeted intervention efforts in areas where the environment intensifies transmission and to support land use activities associated with reduced transmission risk.

705

THE ABILITY OF SEX-SPECIFIC GAMETOCYTE DENSITIES TO PREDICT HUMAN-TO-MOSQUITO TRANSMISSION OF MALARIA PARASITES FROM THE ASYMPTOMATIC RESERVOIR

Derrick Mathias[1], Vincent Nyasembe[2], Brian B. Tarimo[3], Christopher Basham[4], Meredith Muller[4], Mwajabu Loya[5], Zachery Park[4], Feng-Chang Lin[4], Jonathan Juliano[4], Rhoel Dinglasan[2], Billy Ngasala[5], Jessica Lin[4]

[1]*Florida Medical Entomology Laboratory, University of Florida, Vero Beach, FL, United States,* [2]*Emerging Pathogens Institute, University of Florida, Gainesville, FL, United States,* [3]*Vector Immunity & Transmission Biology Unit, Department of Environmental Health & Ecological Sciences, Ifakara Health Institute, Bagamoyo, United Republic of Tanzania,* [4]*Institute of Global Health & Infectious Diseases, University of North Carolina, Chapel Hill, NC, United States,* [5]*Department of Parasitology & Medical Entomology, Muhimbili University of Health & Allied Sciences, Dar es Salaam, United Republic of Tanzania*

In an area of declining endemicity in Tanzania, we are investigating *Plasmodium falciparum* transmission from asymptomatic individuals to *Anopheles gambiae* (IFAKARA strain) via direct skin feeds (DSFs). In Oct-Nov 2018 and Apr-Jul 2019, we used school- and clinic-based recruitment to screen 2,233 asymptomatic volunteers ≥ 6 years of age (range=6-67, median=13), finding *P. falciparum* prevalences of 14% by rapid diagnostic test (RDT), 11% by microscopy, and 27% by 18s rRNA qPCR. We enrolled 206 participants in total, of which 67% were RDT-positive and 31% were positive only by qPCR. Participants were also screened for gametocytes, the transmissible stage to mosquitoes, using two sex-specific RT-qPCR assays: PF3D7_0630000 (female target, limit of detection (LOD)=1 gametocyte/µL) and PfMGET (male target, LOD=0.1 gametocyte/µL). Among participants, female gametocytes were detected in 37% (77/206), male gametocytes in 68% (141/206), and both in 37% (76/206). For transmission studies, 195 DSFs were completed with 177 yielding ≥ 25 mosquitoes for midgut dissection and oocyst enumeration. In total, 53% (94/177) of participants were infectious to at least one mosquito. Of these, 4.3% (4/94) were smear-positive for gametocytes, with RT-qPCR showing 40% (38/94) positive for female gametocytes, 67% (63/177) positive for male gametocytes, and 39% (37/94) positive for both. Thus, neither traditional nor molecular tests for gametocytemia were capable of detecting all infectious individuals. However, further analysis revealed that male gametocyte density (gametocytes/µL) was a significant predictor of the proportion of oocyst-positive mosquitoes (regr. coef.=0.0027, p=0.034), while female gametocyte positivity (presence/absence) was associated with the number of mosquitoes with oocyst infections (IRR=2.23, p=0.001). Our study is ongoing but illustrates the challenge of capturing the asymptomatic infectious reservoir. While female gametocytes were predictive of the "transmission burden" (hosts infectious to multiple mosquitoes), many DSFs with a single mosquito infection occurred when only male gametocytes were detected.

706

MALARIA IS NOT ASSOCIATED WITH DIFFERENCES IN MEASURES OF CHILD GROWTH OR MALNUTRITION IN A COHORT OF ONE-YEAR OLD KENYAN CHILDREN

Katherine R. Sabourin

University of Colorado, Aurora, CO, United States

We aimed to determine whether malaria infection during pregnancy was associated with reduced anthropomorphic measures during early childhood. Pregnant Kenyan women were enrolled at their first antenatal clinic (ANC) visit and followed through delivery. Blood was collected at each ANC visit and tested for *Plasmodium falciparum* DNA by real-time

PCR. Our analyses included children born to human immunodeficiency virus negative mothers with height and length measured at one year of age. We measured anthropomorphic Z-scores using the World Health Organization Anthro Survey Analyser. Student's t-tests were used to compare anthropomorphic Z-scores at one year of age by children ever exposed vs never exposed to malaria in utero. Chi-squared or Fisher's exact test was used to compare number of children with stunting (low length-for-age), wasting (low weight-for-length), and underweight (low weight-for-age) in the two malaria exposures groups. Of the 67 children included in our final analyses 36 (54%) were male and 31 (46%) had a malaria exposure in utero. At one year, 30 (45%) children were stunted (low length-for-age), one (1%) had wasting (low weight-for-length), and four (6%) were underweight (low weight-for-age). We found no significant differences in the Z-scores at one year age for children ever exposed to malaria during pregnancy versus those not exposed to malaria during pregnancy (length-for-age p-value=0.54, weight-for-age p-value=0.92, body mass index-for-age p-value=0.30, weight-for-length p-value=0.90). We found no significant association between stunting (p-value=0.81), wasting (p-value=0.46), or being underweight (p-value=1.00) at one year of age and malaria during pregnancy. Exposure to malaria in utero did not appear to affect measures of child growth and malnutrition at one year of age.

707

ASYMPTOMATIC MALARIA IN LIMPOPO PROVINCE, SOUTH AFRICA: BURDEN OF INFECTION

Mukhethwa Munzhedzi[1], Jennifer L. Guler[2], Darwin J. Operario[2], Piper E. Shifflett[2], Sara Krivacsy[2], Rebecca Dillingham[2], Elizabeth T. Rogawski-McQuade[2], Pascal O. Bessong[1]

[1]*University of Venda, Thohoyandou, South Africa,* [2]*University of Virginia, Charlottesville, VA, United States*

Asymptomatic *Plasmodium* infection is prevalent in endemic areas of Africa; this poses a challenge to malaria prevention and control strategies, particularly in South Africa as it aims to eliminate malaria by 2025. Northeastern South Africa, which encompasses Kruger National Park, is rated a malaria high-risk zone due to favorable climatic conditions (low altitude, high relative humidity, temperature and rainfall) and geographic location (nearby endemic countries). There are approximately 4-5 asymptomatic infections for each clinical infection in endemic areas which potentially seed malaria outbreaks during wet seasons. We aimed to determine the prevalence of asymptomatic *Plasmodium* infection in the Ha-Lambani village in the Vhembe District of Limpopo Province to understand whether these infections can contribute to intermittent outbreaks. In the absence of an outbreak, we collected blood from 333 consenting asymptomatic individuals (204 females and 129 males, ages between 0.5-94 years) from the community. We tested for *P. falciparum* infection using rapid diagnostic tests (RDT) and isolated DNA to perform High Resolution Melt (HRM) analysis to detect infection and determine *Plasmodium* species. The study population had more females infected than males (11,8%, (n=24); 9.3%, (n=12), respectively). Significantly more minors aged ≤17 years were infected compared with adults >17 (n=20 (12.4%), n=16 (9.3%); P=0.0013) respectively. Overall, we report infection in our population of 10.8% (n=36), *P. falciparum* accounted for 4.8% (n=16) by RDT/HRM; and *P. ovale* accounted for 6% (n=20) by HRM respectively. Our findings suggest that the high level of asymptomatic *Plasmodium* infections can be an underappreciated *Plasmodium* reservoir. Perhaps *P. ovale* should also be included in testing. Although RDTs are useful, HRM is better suited for detecting *Plasmodium* infections in malaria endemic areas. Consequentially, malaria control policies should be designed for monitoring and managing malaria infections in asymptomatic carriers.

708

HUMAN MOBILITY AND URBAN MALARIA RISK IN THE MAIN TRANSMISSION HOTSPOT OF AMAZONIAN BRAZIL

Igor C. Johansen, Priscila T. Rodrigues, Rodrigo M. Corder, Marcelo U. Ferreira

University of São Paulo, Sao Paulo, Brazil

Although the overall burden of malaria in Latin America and the Caribbean has decreased dramatically over the past two decades, transmission persists in 21 countries in the region. Brazil accounts for 23% of the cases in the Americas and, in this country, malaria occurs specially in the Amazon, that concentrates 99% of the notifications. Malaria in the Amazon has traditionally been perceived as a disease of the rural poor. Nevertheless, since the mid-1990s it has been increasingly reported within and near urban centers. Here we analyze the case of Mâncio Lima municipality, located in the Juruá Valley, in the Brazilian Amazon. It has currently the highest annual parasite incidence for a municipality in Brazil and its urban area accounts for 49% of the locally acquired malaria cases. Our hypothesis is that population mobility can contribute to the maintenance of this disease in the town. We analyzed two types of mobility. Urban to rural mobility was evaluated using structured questionnaires applied to a household-based random sample of approximately 20% (n=1,903) of the urban residents to obtain sociodemographic data and travel histories. To investigate rural to urban mobility we retrieved all malaria case notifications from the Juruá Valley that were entered into the notification system of the Ministry of Health of Brazil from January 2016 to December 2018. This data allowed us to quantify and locate the origin of the imported malaria episodes into the town of Mâncio Lima. Logistic regression was applied to assess correlates of urban to rural mobility. We found a positive association between participants reporting at least one overnight trip in the previous 12 months and the population aged 16 to 40 years old (OR, 1.79; 95% CI, 1.23-2.61); owing a second residence outside the town (OR, 2.86; 95% CI, 2.24-3.64); being self-employed (OR, 1.39; 95% CI, 1.02-1.88); and occasionally fishing (OR 1.92; 95% CI, 1.49-2.47). We argue that understanding the factors associated to the mobility between urban areas and rural settlements favors the elaboration of more effective public health interventions to face the increasing urban malaria rates in the Brazilian Amazon.

709

ASSESSMENT AND QUANTIFICATION OF TEMPORAL AND SPATIAL CHANGES TO *PLASMODIUM FALCIPARUM* ALLELIC STRUCTURE USING DEEP SEQUENCING

Brian Kabunga Andika[1], Naomi Maina[2], Victor Mobegi[3], Kimita Gathii[1], Josphat Nyataya[1], John Waitumbi[1]

[1]*KEMRI/Walter Reed Project, Kisumu, Kenya,* [2]*Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya,* [3]*Univeristy of Nairobi, Nairobi, Kenya*

Multiplicity of infection (MOI), defined as the number of genetically distinct parasite strains co-infecting a single host is an important indicator of transmission. This study evaluated the utility next generation sequencing (NGS) in elucidating the allelic plasticity of *Plasmodium falciparum* infections. Blood samples that were collected between January 2009 and December 2019 from patients with *P. falciparum* malaria at hospitals in diverse eco-regions of Kenya will be used. The study first determined the lowest parasite density that can provide detectable alleles. For this determination, *P. falciparum* 3D7 culture at varying parasite density was used. This data was then used to select 484 study samples with parasite densities >300 parasites/µL. To further validate the MOI NGS assay with samples of appropriate matrix, 4 patient samples were analyzed. The hypervariable region of *msp1* block 2 was first amplified with primers that target the hypervariable locus of the *K1, MAD20* and *RO33* alleles. The amplicons were then sequenced on an Illumina MiSeq platform and analyzed using the DADA2 and Phyloseq pipelines that utilize a method that infers exact amplicon sequence variants (ASVs) from

high-throughput amplicon sequencing data. A database for *K1, MAD20 and RO33* allele types was used as reference for calling haplotypes. Length polymorphism is the conventional method for scoring haplotypes and is used for differentiating recrudescence from new malaria infections. By this method, the allelic frequencies in the 4 specimens were 6 for K1, 11 for RO33 and 5 for MAD20 with a mean MOI of 3.75. In addition to length polymorphism, NGS allows visualization of sequence polymorphisms that would not be possible by traditional approach. 107 amplicon sequence variants (ASV) were observed: 17 for K1, 5 for MAD20, and 85 for R033. Of the 107 ASVs, 98 had amino acid mutations. NGS allowed 3D visualization of *msp1* haplotypes. The ongoing analysis of malaria parasites from diverse ecoregions of Kenya will establish the usefulness of this new perspective of analyzing *P. falciparum* haplotypes.

710

POOLED DEEP SEQUENCING OF *PLASMODIUM FALCIPARUM* DRUG RESISTANCE GENES *CRT, MDR1, K13, DHPS, DHFR,* AND *CYT-B* USING 450 THERAPEUTIC EFFICACY STUDIES SAMPLES FROM DIOURBEL AND KEDOUGOU SITES IN SENEGAL

Mouhamad Sy[1], Julia Kelley[2], Patel S. Dhruviben[2], Udhayakumar Ventakachalam[2], Eldin Talundzic[2], Daouda Ndiaye[1]

[1]*University Cheikh Anta Diop Dakar (UCAD), Dakar, Senegal,* [2]*Malaria Branch, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, United States*

All populations, including pregnant women, are at risk for the malaria in Senegal. Since 2006 the National Malaria Control Program (NMCP) has recommended artemether-lumefantrine (AL) or artesunate-amodiaquine (ASAQ) as first-line treatment for uncomplicated *P. falciparum* malaria. More recently, in 2017, dihydroartemisinin-piperaquine (DPQ) has been recommended as a second-line treatment. Intermittent preventive treatment with sulfadoxine-pyrimethamine (IPTp-SP) is recommended for reducing the risk of malaria in pregnancy. To date artemisinin combination therapies, AL, ASAQ, DPQ and IPTp-SP remain efficacious in Senegal. However, resistance mutations in *k13, mdr1, crt, dhps* and *dhfr* threaten the the efficacy of these treatments for vulnerable populations in Senegal. Therefore, in addition to routine therapeutic efficacy studies (TES), molecular surveillance for drug resistant markers in these genes is an important public health activity.Towards this end, using 450 *P. falciparum* samples from Diourbel and Kedougou as part of a TES were genotyped using the pooled Malaria Resistance Surveillance (MaRS) next generation sequencing (NGS) protocol. Briefly, the 450 samples were pooled in 10:1 pools and the full-length drug resistance genes *crt, mdr1, k13, dhps, dhfr,* and *cyt-b* were assessed for known and other putative molecular markers of resistance. Data analysis was performed using the MaRS Next-generation Sequence-analysis Toolkit (NeST) and validated using Geneious Prime . The following known drug resistant mutations were found in the 45 pools: *crt (*74I , 76T , 220S, 271E, 356T and 371I), *mdr1* (86Y, 184F), *dhfr* (51I, 59R, 108N) *dhps* (536A, 437G, 540E, 581G, 613S*)* with different allele frequency (AF). No drug resistant mutations were found in *k13* and *cytb*. In addition, the following non-drug resistance associated mutations were identified: 10 in mdr, 16 in crt, 17 in dhfr, 7 dhps, 22 in k13 and 31 in cytbThe pooled MaRS protocol provides a rapid targeted deep amplicon sequencing approach for screening large number of samples for known and other putative molecular markers of resistance in population-based studies.

711

PREVALENCE OF *PLASMODIUM FALCIPARUM* DIHYDROFOLATE REDUCTASE *(PFDHFR)* MUTANT HAPLOTYPES IN KIFFA, SOUTH-CENTRAL MAURITANIA

Mohamed salem Ahmedou salem[1], Mariem Bowbe Cherif[1], Nasserdine Papa Mze[2], Boushab Mohamed Boushab[3], Hervé Bogreau[4], Leonardo Basco[5], Ali Ould Mohamed Salem Boukhary[1]

[1]*Université de Nouakchott Al-Aasriya, Nouakchott, Mauritania,* [2]*Aix-Marseille université, Marseille, France,* [3]*Department of Internal Medicine and Infectious Diseases, Kiffa Regional Hospital, Kiffa, Mauritania,* [4]*Unité Parasitologie et Entomologie, Département des maladies infectieuses, Institut de Recherche Biomédicale des Armées, Marseille, France, Marseille, France,* [5]*Aix-Marseille Univ, IRD, AP-HM, SSA, VITROME - IHU-Méditerranée Infection, Marseille, France*

Sulfadoxine-pyrimethamine (SP) is the drug of choice to prevent pregnancy-associated malaria. Resistance of *Plasmodium falciparum* to SP is associated with the occurrence of mutations on *P. falciparum* dihydrofolate reductase (*Pfdhfr*) and dihydropteroate synthetase (*Pfdhps*) genes. The objective of the study was to determine the prevalence of *Pfdhfr* mutations associated with pyrimethamine resistance in *Plasmodium falciparum* isolates from Kiffa, south-central Mauritania, where epidemiological data are lacking.Febrile patients consulting at the hospital center of Kiffa were screened for the presence of malaria parasites using Falcivax™ rapid diagnostic test and thick blood film between September and October 2018. Malaria diagnosis was further confirmed by a species-specific nested PCR. *Pfdhfr* point mutations were analyzed by nested PCR followed by Sanger DNA sequencing. A total of 77 febrile patients were screened for malaria. The presence of malaria parasites was confirmed by PCR in 71/77 (92.2%). The only malaria species identified was *Plasmodium falciparum.* The *Pfdhfr* gene was successfully amplified and sequenced in 75.5% (37/49) of the samples. The prevalence of *Pfdhfr* mutations N51I, C59R, and S108N was 75.6% (28/37), 78.4% (29/37), and 81.1% (30/37), respectively. The triple mutation CIRNI was observed in 67.6% (25/37). No mutation was observed at codons 16 and 164.The present study showed the high prevalence of triple mutants CIRNI, suggesting a high level of resistance pattern to pyrimethamine in the study site, as observed in the vast majority of African countries.

712

EVALUATION OF *PLASMODIUM FALCIPARUM* HISTIDINE-RICH PROTEIN 2 AND 3 (*PFHRP*2 AND *PFHRP*3) GENE POLYMORPHISMS IN KENYA

Martha N. Kivecu[1], Brenda M. Mugambi[2], Victor Mobegi[3], Benjamin Opot[1], Dennis Juma[1], Hosea Akala[1], Ben Andagalu[1], Amanda Roth[1]

[1]*Us Army Medical Research Directorate- Africa, Kenya (USAMRD-A), Kisumu, Kenya,* [2]*Centre for Biotechnology and Bioinformatics (CEBIB), University Of Nairobi, Nairobi, Kenya, Nairobi, Kenya,* [3]*Biochemistry Department University of Nairobi, Nairobi, Kenya*

Globally, about 75% of all malaria suspected cases are diagnosed by malaria rapid diagnostic tests (RDTs). The accuracy of the most commonly used *Plasmodium falciparum* histidine-rich protein 2 (*Pfhrp*2) based RDTs can be impaired by either deletion in the *Pfhrp2* gene or cross-reaction with *Pfhrp3* antigens. The World Health Organisation (WHO) criteria that require greater than 95% accuracy as the threshold for selection or withdrawal of RDTs argue for active mapping of *Pfhrp2* and *Pfhrp3* genes deletion. This study investigated the prevalence of *Pfhrp2* and/or *Pfhrp3* genes deletion in three of five malaria transmission regions in Kenya. Generally, 350 samples comprising 255 collected between 2013 and 2017 (post-RDTs) and 95 collected between 2003 and 2005 (pre-RDTs) were diagnosed for malaria by microscopy, *Pfhrp*2/pan (*Plasmodium* Lactate Dehydrogenase enzymes) Parascreen™ RDT and 18S rRNA PCR. For the 95 pre-RDTs samples RDT was not done. All *P. falciparum* PCR positive samples with at least 30 parasites per microliter were genotyped using primers targeting *Pfhrp*2 and *Pfhrp*3 encoding genes to identify genes

deletions. The sensitivity of *Pfhrp*2 based RDT was 89.2% in reference 195 pan and *P. falciparum* PCR positive samples. Of 195 samples 21 (10.8%) were negative by *Pfhrp2* based RDT. All the 21 false RDT negative samples had *Pfhrp*2 and only one sample was negative for the *Pfhrp*3 gene. There was no clear temporal or spatial trend of false-negative RDTs results due to incomparable sample size among sites across the study period. The prevalence of *Pfhrp*2 gene deletion in *P. falciparum* PCR positive samples (n=317) was 0.3% while that of the *Pfhrp*3 gene was 1.3%. There was no significant correlation between Both *Pfhrp*2 and *Pfhrp*3 genes deletion and any of their flanking gene deletion (P.value greater than 0.005). This study shows false-negative RDTs are present, however, they could not be attributed to *Pfhrp*2 and/or *Pfhrp*3 gene deletion. This finding heralds the need for investigating additional mechanisms of false-RDTs negativity. Further work needs to involve a concurrent cohort as well as investigate new RDTs targets in search of more reliable RDTs.

713

QUANTIFYING *PLASMODIUM FALCIPARUM* PARASITE DIVERSITY AND POPULATION CONNECTIVITY USING GENOMIC DATA ACROSS A TRANSMISSION GRADIENT IN ZAMBIA

Abebe A. Fola[1], Daniel J. Bridges[2], Jack Dorman[1], Ilinca Ciubotariu[1], Mulenga C. Mwenda[2], Brenda Mambwe[2], Conceptor Mulube[2], Moonga Hawela[3], Douglas E. Norris[4], William J. Moss[5], Giovanna Carpi[1]

[1]*Department of Biological Sciences, Purdue University, West Lafayette, IN, United States,* [2]*PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Lusaka, Zambia,* [3]*National Malaria Elimination Centre, Zambia Ministry of Health, Chainama Hospital Grounds, Lusaka, Zambia,* [4]*W. Harry Feinstone Department of Molecular Microbiology and Immunology, The Johns Hopkins Malaria Research Institute, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [5]*Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States*

Zambia is a malaria-endemic country and suffers a high *Plasmodium falciparum* malaria burden with approximately 5 million reported cases per year. As a result of intervention scale-up, the country has reported a significant national reduction in malaria prevalence from 19% in 2015 to 9% in 2018 in children under 5 by microscopy. This reduction however is associated with an increasingly heterogeneous spatial pattern of transmission, with *P. falciparum* prevalence ranging at the provincial level from 0.1% to 30.4%. As elimination efforts advance, additional metrics e.g. estimates of multiplicity of infection, population structure and genetic relatedness between populations and across different epidemiological zones may be critical to understanding transmission and responding appropriately. This project aimed to quantify parasite diversity and the spatial scale of parasite connectivity by examining *P. falciparum* genomes across Zambia using samples collected in the 2018 Zambia National Malaria Indicator Survey. Using a multiplexed hybrid capture technique to selectively enrich and deep sequence whole *P. falciparum* genomes directly from ~400 dried-blood samples, we present *P. falciparum* whole genome sequence data from phase 1 of this effort. Across the sequenced samples (n = 26) from two high prevalence neighboring provinces, we obtained a median of 72.4% of *P. falciparum* genomes covered at >20x. We identified a high proportion of *P. falciparum* mixed infections based on genome-wide signal (Fws < 0.95). We found previously undocumented levels of genome-wide differentiation, with 30,436 identified SNPs (MAF>0.05) among the analyzed samples. Preliminary analysis of identity-by-descent (IBD)-based relatedness between pairs of major haplotypes within samples revealed that 27% of pairs shared >90% of their genome, reflecting relatively low genetic relatedness. The findings from this project shed light on the extent of *P. falciparum* genomic variation and spatial transmission patterns within and between different epidemiological zones, which can inform malaria control and help sustain progress toward malaria elimination in Zambia.

714

MOLECULAR SURVEILLANCE OF THE POPULATION DIVERSITY AND ARTEMISININ DRUG RESISTANCE GENE (KELCH-13) OF *PLASMODIUM FALCIPARUM* IN NIGERIA

Fehintola Victoria Ajogbasile, Paul Eniola Oluniyi, Adeyemi Tolulope Kayode, Kazeem Akano, Jessica Nnenna Uwanibe, Philomena Eromon, Onikepe Folarin, Christian Happi

Redeemer's University, Ede, Nigeria

Malaria remains a public health burden especially in Nigeria. To develop new control and elimination strategies or refine existing ones, it is important to understand parasite population diversity, transmission patterns and mechanisms of drug resistance. In this study, we aimed at characterizing the population diversity of *Plasmodium falciparum* in Nigeria, using 12 microsatellite loci. We also used targeted sequencing to investigate the status of the *P. falciparum* Kelch 13 (K-13) gene. Genomic DNA was extracted from 300 dried blood spot filter paper samples (from three States; Kano, Enugu and Plateau States of Nigeria) using Zymo DNA extraction kit. A semi-nested PCR amplification was used to amplify 12 microsatellite loci of *P. falciparum,* while the K-13 propeller gene was amplified by nested PCR. Microsatellite fragment sizes were analyzed using GeneMapper and GENALEX 6.5 and K-13 amplicons were sequenced using Applied Biosystems 3500XL series Genetic Analyzer to detect known and novel polymorphisms in the gene. The consensus sequences of samples were mapped with the reference gene sequence obtained from NCBI and a maximum likelihood tree using IQTREE v1.6.1 was inferred. The mean COI for 12 microsatellites loci were 1.78 (Kano), 2.73 (Enugu) and 2.73 (Plateau). The expected heterozygosity (He) was similar across the all States, with mean He values across all loci of 0.824 (Kano), 0.821(Enugu) and 0.788 (Plateau). These results suggest high parasite endemicity in these States of Nigeria. Also, AMOVA value was 0.175 (P=0.001) while the overall linkage disequilibrium for all States was 0.0145. The combined PCA plot showed genetic similarities amongst these States. These results showed low levels of population divergence. Out of the 13 polymorphisms detected in the K-13 gene, two (A557S and A578S) were previously reported in other endemic countries. None of the observed polymorphisms in Nigerian samples have been validated to contribute to ART resistance but two (G449S and V487E) were deleterious. Phylogenetic analysis revealed close clustering of all our sequences in the same clade showing limited diversity of the K-13 gene in Nigeria.

715

MONITORING THE DYNAMICS OF *PLASMODIUM FALCIPARUM* POLYCLONALITY AND PFCSP GENE DIVERSITY IN RESPONSE TO RTS,S USING NEXT-GENERATION SEQUENCING

Cheikh C. Dieng[1], Karen Lopez[1], Jordan Connors[1], Jordan Connors[1], Denis Machado[1], Shittu B. Dhikrullahi[2], Dickson Donu[2], Daniel A. Janies[1], Linda E. Amoah[2], Yaw A. A. Afrane[2], Eugenia Lo[1]

[1]*UNC Charlotte, Charlotte, NC, United States,* [2]*University of Ghana, Acra, Ghana*

Recently, there has been a resurgence of malaria in several African countries. Over 80% of malaria deaths occur in children under 5 years of age. Malaria control strategies have been progressively shifted to specific populations and/or areas to maximize effectiveness. Ghana is one of the three African countries where the world's first malaria vaccine, RTS, S, is recently launched. The vaccine contains part of the central repeat region and the complete C-terminal of the circumsporozoite protein (CSP) gene of the 3D7 strain. Polymorphism in the *PfCSP* protein has been reported from several parts of the world. However, whether RTS, S-induced immunity is *PfCSP* allele-dependent and if selection favors non-3D7 strains are unclear. This study aims to examine the genetic polymorphism of the *PfCSP* genes in clinical *lasmodium. falciparum* cases and provide a baseline of parasite diversity prior to vaccine implementation in Ghana. A total of 212 clinical samples were collected from Seikwa located in the Brong Afrong region where the vaccine is currently being deployed. Preliminary

data indicated a high rate of polyclonal infections, with up to 3 clones being detected per sample based on the allele frequency among mapped reads. Parasite clones detected within the same host were not genetically similar to one another. Instead, they were distributed in various subclades and closely related to clones identified from other hosts. A small portion of the polyclonal infections contained the 3D7 strain. Our broader analyses of 233 Ghanaian *P. falciparum* genomes obtained from the Pf3k database (MalariaGen) revealed that 12% of the isolates resembled the 3D7 strain at the *PfCSP* Th2R and Th3R regions. A total of 58 Th2R and 17 Th3R unique haplotypes were detected in the 233 Ghanaian *P. falciparum* samples, yielding a nucleotide diversity of 18% across all sites. It is yet to be investigated if the high *PfCSP* haplotype diversity and low resemblance to the 3D7 strain have an impact on the anti-*CSP* immune response and thereby the efficacy of RTS, S.

716

A CAPTURE PROBE-BASED APPROACH REVEALS SEVERE MALARIA-ASSOCIATED PFEMP1 DOMAINS EXPRESSED IN UNCOMPLICATED MALARIA AMONG MALIAN CHILDREN

Emily Stucke[1], Antoine Dara[2], Drissa Coulibaly[2], Abdoulaye K. Kone[2], Karim Traoré[2], Bouréima Guindo[2], Bourama M. Tangara[2], Amadou Niangaly[2], Modibo Daou[2], Issa Diarra[2], Youssouf Tolo[2], Mody Sissoko[2], Albert E. Zhou[1], Matthew B. Laurens[1], Amed Ouattara[1], Bourema Kouriba[2], Ogobara K. Doumbo[2], Shannon Takala-Harrison[1], David Serre[1], Christopher V. Plowe[3], Mahamadou A. Thera[2], Joana C. Silva[1], Mark A. Travassos[1]

[1]*University of Maryland School of Medicine, Baltimore, MD, United States,* [2]*Malaria Research and Training Center, University of Sciences, Techniques and Technologies, Bamako, Mali,* [3]*Duke Global Health Institute, Duke University, Durham, NC, United States*

Plasmodium falciparum membrane protein-1 (PfEMP1) antigens are encoded by the *var* gene family, of which there are ~60 per parasite genome. Only one PfEMP1 is displayed on the surface of an infected red blood cell, where it can bind host cell receptors and mediate cytoadhesion. Partial protection from clinical malaria has been associated with antibodies to variant surface antigens such as PfEMP1. Expression of certain PfEMP1s, particularly those binding the human receptors endothelial protein C receptor (EPCR), intercellular adhesion molecule 1 (ICAM1), and chondroitin sulfate A, have been associated with severe malaria. Using custom-designed capture probes, we sequenced PfEMP1 RNA from three severe and nine uncomplicated *P. falciparum* infections in rural Malian children, and quantified *var* gene expression using *de novo* assembled and genomic *var* sequences. For the most abundant transcripts, we classified expressed PfEMP1 domains to determine potential binding phenotypes. All infections included expression of CD36-binding PfEMP1 transcripts (12/12), and the majority of both severe (2/3) and uncomplicated malaria (5/9) infections included ECPR-binding PfEMP1 transcripts. Most severe malaria infections (2/3) included expression of ICAM1-binding and dual receptor-binding PfEMP1s. We also observed expression of ICAM1-binding PfEMP1s in most uncomplicated malaria infections (7/9), while only a few included expression of EPCR/ICAM1 dual-binding PfEMP1s (2/9). Interestingly, in one uncomplicated malaria infection, a dual-binding PfEMP1 transcript predominated, while in three uncomplicated malaria infections, a *var2csa* transcript was predominant. These results suggest the need to consider host factors, including acquired immunity, hemoglobinopathies, and potentially variation in human cell receptors, in understanding PfEMP1 virulence. We are applying these methods to a larger severe malaria case-control study to determine the PfEMP1 structure and common domains expressed in cases of severe malarial anemia and cerebral malaria *versus* matched uncomplicated malaria controls.

717

A HIGH-THROUGHPUT PHENOTYPIC SCREEN UNRAVELS *PLASMODIUM FALCIPARUM* GENES ESSENTIAL FOR MALARIA TRANSMISSION (GAMETOCYTE DEVELOPMENT)

Jyotsna Chawla[1], Jenna Oberstaller[1], Min Zhang[1], Chengqi Wang[1], Shulin Xu[1], Anatoli Naumov[1], Andreas Seyfang[1], Thomas D. Otto[2], Julian C. Rayner[3], John Adams[1]

[1]*Center for Global Health and Infectious Diseases Research, University of South Florida, Tampa, FL, United States,* [2]*Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom,* [3]*Cambridge Institute for Medical Research, University of Cambridge, Cambridge, United Kingdom*

Malaria transmission of the causative deadly parasite *Plasmodium falciparum* is mediated by mature sexual forms called gametocytes that represent an infection bottleneck. With restricted use of drugs targeting this stage, gametocytes propagate through the population unchecked, making it a prime target to block human to vector transmission. Previous work in our laboratory achieved saturation-level mutagenesis of *P. falciparum* (>38,000 insertions) and determined essentiality of genes for asexual blood-stage growth under ideal *in vitro* conditions. In this study, our forward genetic approach was extended to assess the likely importance of asexual dispensable genes in sexual-stage development. We developed a high-throughput gametocyte screening protocol using a pilot library of 128 unique *piggyBac* mutant clones. The preliminary study resulted in two distinct phenotypes that are 'Advantageous' or 'Deleterious' to gametocyte development. Next, we performed a large-scale screen with a 600-mutant library and identified early and late gametocyte essential genes. These genes are characterized further for their expression patterns, GO pathways, conservation with other *Plasmodium spp.* and other identifiable features. Our goal is to delineate the pathways and processes critical for development and maturation of infectious male and female gametocytes. Through this study, we anticipate closing an important gap in the *P. falciparum* life cycle and lay the foundation for new antimalarial transmission-blocking intervention strategies.

718

CHARACTERIZATION OF GENES IN *PLASMODIUM FALCIPARUM* MUTANTS ASSOCIATED WITH ALTERED SENSITIVITY TO ARTEMISININ

Caroline Simmons[1], Jenna Oberstaller[1], Min Zhang[1], Shulin Xu[1], Justin Gibbons[1], Chengqi Wang[1], Deborah Casandra[1], Andreas Seyfang[1], Thomas D. Otto[2], Julian C. Rayner[3], John H. Adams[1]

[1]*University of South Florida, Tampa, FL, United States,* [2]*University of Glasgow, Glasgow, United Kingdom,* [3]*University of Cambridge, Cambridge, United Kingdom*

Artemisinin combination therapies (ACTs) have led to a significant decrease in *Plasmodium falciparum* global malaria mortality, however, emerging resistance to ACTs in Southeast Asia threatens to reverse these gains. To address the lack of functionally annotated genes in the *P. falciparum* genome, our laboratory adapted the *piggyBac* forward genetic method for characterizing *P. falciparum* genes. This approach relies on the single insertion genomic disruptions in our *piggyBac* mutants to elucidate genotype-phenotype associations in *P. falciparum*. Chemogenomic profiling revealed some *piggyBac* mutants have altered sensitivities to artemisinin (ART) and other mutants have altered sensitivity to other antimalarial compounds. In a previous transcriptome study of a *P. falciparum* K13 *piggyBac* mutant, we identified the interacting molecular processes of dysregulated K13 gene expression that ultimately shortened early intraerythrocytic development and led to increased ART drug sensitivity. In this study, we have further elucidated changes in other ART-sensitive mutants that may have a role in altering the parasite's drug response by characterizing additional *piggyBac* mutants through RNAseq at varying timepoints during the intraerythrocytic asexual development cycle. These findings will contribute to a more comprehensive understanding of the molecular activities that are altered in *P. falciparum* in

response to ART and other antimalarial drugs. Our goal for chemogenomic profiling is to elucidate a comprehensive molecular network of antimalarial drug activity to provide a better understanding of artemisinin mechanism of action and the parasite's processes affecting drug sensitivity and resistance mechanisms.

720

POPULATION GENOMICS IDENTIFIES A DISTINCT *PLASMODIUM VIVAX* POPULATION ON THE CHINA-MYANMAR BORDER

Awtum Marie Brashear[1], Qi Fan[2], Yubing Hu[3], Yuling Hu[3], Yan Zhao[3], Zenglei Wang[4], Yaming Cao[3], Jun Miao[1], Alyssa Barry[5], Liwang Cui[1]

[1]*University of South Florida, Tampa, FL, United States,* [2]*Dalian Institute of Technology, Dalian, China,* [3]*China Medical University, Shenyang, China,* [4]*Chinese Academy of Medical Sciences and Beijing Union Medical College, Beijing, China,* [5]*Deakin University, Melbourne, Australia*

Plasmodium vivax has become the predominant malaria parasite and a major challenge for malaria elimination in the Greater Mekong Subregion (GMS). Yet, our knowledge about the evolution of *P. vivax* populations in the GMS is fragmental. Malaria cases on the China-Myanmar border are increasingly caused by *P. vivax* and the uneven malaria incidence between Myanmar and China makes malaria in this region interesting. We performed whole genome sequencing on 21 *P. vivax* samples from the China-Myanmar border (CMB) and compared them to over 200 samples from the rest of the GMS. SNP-based admixture and phylogenetic analyses demonstrated that parasites on the CMB are distinct from the rest of the Greater Mekong Subregion. The CMB parasites displayed a higher proportion of monoclonal infections, and high identity by descent, indicating a monoclonal expansion resulting from the *P. vivax* outbreaks occurring during this study period. We further characterized this population using genome-wide scans for selection and constructing *de novo* assemblies of 4 high quality isolates which showed high diversity in multi-gene families.

721

MECHANISTIC MALARIA MODEL PREDICTIONS OF GENETIC FEATURE VARIATION ACROSS TRANSMISSION SCALES QUALITATIVELY MATCH OBSERVED DATA FROM 27 SITES THROUGHOUT SENEGAL IN 2019

Albert Lee[1], Katherine E. Battle[1], Jessica Ribado[1], Jonathan Russell[1], Aida Badiane[2], Yaye Dié Ndiaye[3], Awa Deme[2], Fatou Ba Fall[4], Médoune Ndiop[4], Alioune B. Gueye[4], Ibrahima Diallo[4], Rachel Daniels[5], Stephen Schaffner[5], Bronwyn MacInnis[5], Daniel Hartl[6], Doudou Sène[4], Daouda Ndiaye[2], Edward Wenger[1], Dyann Wirth[7], Sarah Volkman[7], Caitlin Bever[1], Joshua L. Proctor[1]

[1]*Institute for Disease Modeling, Bellevue, WA, United States,* [2]*Université Cheikh Anta Diop, Dakar, Senegal,* [3]*Hôpital Aristide Le Dantec, Dakar, Senegal,* [4]*Programme National de Lutte contre le Paludisme, Dakar, Senegal,* [5]*Broad Institute of MIT and Harvard, Cambridge, MA, United States,* [6]*Harvard University, Cambridge, MA, United States,* [7]*Harvard T.H. Chan School of Public Health, Boston, MA, United States*

Malaria genomic data is emerging as a critical tool for adding specificity to transmission patterns and increasing the value of routine surveillance efforts by national malaria control programs. Currently, research institutions and national malaria control programs in endemic countries are scaling up malaria genomic surveillance to demonstrate the programmatic value of genetic data for a wide variety of use-cases, including inferring transmission patterns and identifying local vs. imported transmission. Mechanistic transmission models offer a framework for testing and validating epidemiological relationships between genetic data and routine surveillance measures. Here, we describe the generalization of a previous molecular barcode model to enable characterization of a wider range of malaria transmission and inclusion of more genomic sequence information including sequencing data. Using simulation, we demonstrate how features derived from synthetic genomes, such as complexity of infection and clonal expansion, are related to transmission properties. We then compare these model predictions to a set of malaria parasite genetic barcodes collected from 26 health posts across the transmission zones in Senegal during the 2019 transmission season. The model predictions are qualitatively similar to the observed genetic data when comparing metrics complexity of infection and the number of repeated barcodes, with Spearman correlations up to 0.5; moreover, quantifying the uncertainty from the stochastic simulations and correlating with the variation in the observed data allows for an assessment of sampling and non-sampling uncertainty. Using this model framework informed by pan-Senegal data, we develop a suite of temporal and spatial genetic features that provide information on linkage between and within regions. We demonstrate how a feature selection procedure can identify the most informative features depending on the use-case. More broadly, we believe modeling efforts will play a key role in understanding how to most efficiently and effectively integrate malaria genomic data into routine malaria surveillance programs.

722

MATERNAL TRANSFER AND ACQUISITION OF MALARIA SPECIFIC ANTIBODIES TO PFEMP1 IN THE FIRST YEAR OF LIFE

Azizath Moussiliou[1], Louise Turner[2], Nadine Fievet[3], Achille Massougbodji[1], Thor Theander[2], Philippe Deloron[3], Thomas Lavstsen[2], Nicaise Tuikue Ndam[3]

[1]*Institut de Recherche Clinique du Bénin, Universitéd'Abomey-Calavi, Cotonou; Benin, Cotonou, Benin,* [2]*Centre for Medical Parasitology, University of Copenhagen, Copenhagen, Denmark,* [3]*Université de Paris, MERIT, IRD, F-75006 Paris, France., Paris, France*

The cytoadherence properties of *Plasmodium falciparum* infected erythrocytes (IE) represent a major contributor to the pathogenesis of malaria through interactions with various endothelial cell surface receptors. These interactions are mediated by members of the highly variable *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) expressed on the IE surface. One particular component of PfEMP1 proteins, the cysteine-rich interdomain region (CIDR), is known to play a very important role in the adhesive interactions between IE and endothelial receptors, making this region a potential vaccine target of interest. Here, we investigated the dynamics of maternally-transferred IgG antibodies targeting the CIDR of a panel of different PfEMP1 proteins, as well as infants' own acquisition of antibodies with the same specificities during the first year of life. We used plasma samples collected longitudinally from the offspring of a cohort of pregnant women who had themselves been followed closely through pregnancy. We show that antibodies to all PfEMP1 types declined at similar rates to the point of disappearance over the first six months of life. At 12 months, children had acquired antibody to all types of CIDR domains, mostly in children with documented *P. falciparum* infections. Higher IgG levels to EPCR-binding CIDRα1 variants positively correlated with the timing of first infections. Antibodies to CIDRα1 domains were more frequent in cord blood compared to antibodies to CIDRα2-6 domains. The data presented here show that children develop IgG antibodies to all types of PfEMP1, excluding VAR2CSA, in response to infections during their first year of life. Children anti-PfEMP1 repertoire is built accordingly when the maternal antibodies decrease and the timing of the first infection is more influenced by the immune status of their mothers.

723

CHARACTERIZATION OF NOVEL *PLASMODIUM* SEXUAL STAGE ANTIGENS AS TARGETS OF TRANSMISSION-REDUCING IMMUNITY

Michelle K. Muthui[1], Fiona Angrisano[2], Kataryzna Sala[3], Eizo Takashima[4], William Muasya[1], Brian Omondi[1], Christine K. Kinya[1], Kazutoyo Miura[5], Andrew M. Blagborough[6], Philip Bejon[1], Melissa C. Kapulu[1]

[1]*KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya,* [2]*Imperial College London, London, United Kingdom,* [3]*The Francis Crick Institute,*

London, United Kingdom, [4]Proteo-Science Center, Ehime University, Matsuyama, Japan, [5]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [6]Department of Pathology, University of Cambridge, Cambridge, United Kingdom

To achieve malaria elimination, both scaling up the use of existing malaria control tools and developing new interventions will be required. Transmission-blocking vaccines (TBVs) are 'novel interventions' that seek to curb transmission by reducing the ability of individuals to infect mosquitoes. Current TBV candidates are limited to the gametocyte antigens Pfs230, Pfs48/45 and the gamete antigen Pfs25, prioritised for initial identification rather than efficacy. Pfs25, currently in clinical trials, has shown relatively poor long-term immunogenicity requiring high antibody titres for effective blocking activity. Additionally, though Pfs230 and Pfs48/45 are further down the development pipeline, studies have demonstrated naturally acquired transmission-reducing immune responses (NA-TRI) to both antigens. However, recognition of Pfs230 and Pfs48/45 does not always correlate with transmission reducing activity. Furthermore, NA-TRI exists in the absence of responses to Pfs230 and Pfs48/45. Other gametocyte antigens must therefore play a role in transmission-reducing immunity. We sought to identify novel antigens on the surface of *Plasmodium* gametocytes, gametes and ookinetes with potential as TBV candidates. Using bioinformatic tools, we identified novel and uncharacterised antigens for evaluation and produced them as recombinant protein. These were 9 gametocyte antigens (7 antigens with 2 variant antigens - based on sequences to 3D7 and a Kenyan clinical isolate), and 5 gamete and ookinete-stage candidate antigens. We raised antibodies in mice to these antigens and tested their functional transmission-reducing activity using membrane feeding assays and ookinete conversion assays. Furthermore, we measured immune responses to the identified gametocyte antigens using sera from malaria-exposed individuals. We did this to better understand the dynamics of naturally acquired immune responses to gametocytes and identify biomarkers of recent gametocyte exposure. Results from these experiments will be presented which will improve our understanding of NA-TRI and also identify novel TBV candidate antigens.

724

OPSONIC PHAGOCYTOSIS OF MEROZOITES IS ASSOCIATED WITH PROTECTION AGAINST CLINICAL MALARIA IN GHANAIAN CHILDREN AND IMPLICATES $MSP1_{19}$ AS A POTENTIAL TARGET.

Eric Kyei-Baafour[1], Kwadwo A. Kusi[1], Fareed K. Arthur[2], Regis W. Teindrebeogo[3], Eunice Owusu-Yeboa[1], Michael Theisen[3], Daniel Dodoo[1], Bright Adu[1]

[1]*Noguchi Memorial Insitute for Medical Research, University of Ghana, Legon-Accra, Ghana, [2]Department of Bochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, [3]Centre for Medical Parasitology at Department of International Health, Immunology and Microbiology, University of Copenhagen and Department of Infectious Diseases, Copenhagen, Denmark*

Proteins on the surfaces of free merozoites of *Plasmodium falciparum* are likely targets of functional antibodies. Studies have linked opsonizing antibodies killing *P. falciparum* parasites through Fc receptors in corporation with polymorphonuclear cells as one of the naturally acquired immune mechanisms. Total IgG, responses to crude schizont extract and $MSP1_{19}K$ were measured using indirect ELISA in Ghanaian children (aged 0.5 to13 years, N=219) in a longitudinal malaria cohort study. Ability of the antibodies to opsonize merozoites was measured in plasma using a flow cytometry-based opsonic phagocytosis (OP) assay. Children with high (≥ median) OP index were protected against malaria compared to those with low (< median) OP index (HR 0.60; 95%CI = 0.40-0.90, *p=0.013*) after correcting for the covaraites age, bednet use, and gender. After adjusting for covariates, protected children had higher IgG against crude schizont antigens (β=0.33, 95%CI = 0.04-0.16, p=0.025) and $MSP1_{19}K$ (β=0.38, 95%CI = 0.04-0.71, p=0.029) compared to those susceptible. OP increased significantly (β=2.65, 95%CI = 0.50-4.80, p=0.017) with $MSP1_{19}K$ IgG. This data corroborates previous report on OP of merozoites and finds $MSP1_{19}K$ to be an important target in OP.

725

REGULATION OF CGAS-MEDIATED DNA SENSING PATHWAY BY 2'-5'-OLIGOADENYLATE SYNTHASE LIKE PROTEIN DURING *PLASMODIUM FALCIPARUM* INFECTIONS

Collins Misita Morang'a[1], Nancy Nyakoe[1], Dominic S. Amuzu[1], Jonas Kengne-Ouafo[1], Joe Mutungi[1], Saikou Y. Bah[2], Yaw Bediako[1], Thomas D. Otto[3], Lucas Amenga-Etego[1], Gordon A. Awandare[1]

[1]*WACCBIP, Department of Biochemistry, University of Ghana, Accra, Ghana, [2]Florey Institute, Molecular Biology and Biotechnology, University of Sheffield, Sheffield, United Kingdom, [3]Institute of Infection, Immunity & Inflammation, MVLS, University of Glasgow, Glasgow, United Kingdom*

Understanding the molecular mechanisms involved in the regulation of innate immune responses during *Plasmodium falciparum* infections can facilitate the development of new immunotherapeutics. The objective of this study was to determine the effect of regulation of 2'-5'-oligoadenylate synthase-like protein (OASL) on the production of type I interferons of the human innate immune system. Peripheral mononuclear cells (PBMC) were collected from malaria infected individuals and transfected with three Dicer-Substrate 27-mer duplexes selected from a predesigned set of duplexes from human genome that target the *OASL* gene. The cells were incubated in 5 % CO_2 incubator, then *P. falciparum* AT-rich motif was transfected to stimulate the cyclic GMP-AMP synthase (cGAS) cytosolic sensor to produce type I interferons. PBMCs were stimulated for 24 hours, harvested and RNA was extracted. Real time PCR was used for gene expression analysis of the *OASL, cGAS*, and interferon beta (*IFN*-β) genes using the delta CT method, with *GAPDH* as the endogenous control. A fluorescent-labelled control, which contains a red TYE-563 fluorescence, confirmed successful transfection of siRNA duplexes into PBMCs. Three siRNA duplexes showed transient inhibition of *OASL* expression. Knockdown of *OASL,* followed by stimulation of cGAS sensor using *P. falciparum* AT-rich motif, did not upregulate *OASL* gene levels. This corresponded with significant upregulation of the *IFN*-β levels ($P < 0.05$). In the absence of *OASL* knockdown, the cells showed significant downregulation of *IFN*-β levels ($P < 0.05$). This is the first report on the mechanism by which *OASL* regulates the innate immune response through a feedback mechanism that downregulates production of *IFN*-β. This offers the potential to develop immunotherapeutics to prevent *Plasmodium* DNA mediated inflammatory responses in clinical malaria.

726

THE ROLE OF THERMONEUTRALITY ON THE INNATE IMMUNE SYSTEM OF A MALARIAL MURINE MODEL

Fiorella Vialard, Martin Olivier

Research Institute of the McGill University Health Centre, Montreal, QC, Canada

The fever paroxysm observed in malaria patients occurs when the schizont of *Plasmodium* parasites within infected red blood cells (iRBCs) causes their lysis, triggering immune cells to release inflammatory cytokines. One study has shown that human monocytes stimulated with *Plasmodium falciparum* lysed iRBCs showed a different TNF-α expression pattern than those treated with intact iRBCs. Most mouse model studies are conducted on animals housed at a temperature below their thermoneutral temperature (TT). Suboptimal temperature (ST) is known to affect the mouse immune system but has been poorly investigated in the context of parasitic infections. We hypothesized the inflammatory response (IR) triggered by lysed iRBCs to be significantly different in a murine model at TT compared to ST. To investigate this, we first lysed *Plasmodium berghei* ANKA iRBCs by sonication and used the lysate to stimulate LM-1 macrophages while controlling for several variables. The mRNA levels of candidate cytokines (CCL-2, CCL-3, CCL-4, CXCL-2, IL-6, IL-1β and TNF-α) were measured

by RT-qPCR. We observed modulation of their expression after a 6-hour stimulation with 10^7 lysed iRBCs. We subsequently inoculated groups of mice housed at ST with either PBS, LPS, lysed/intact iRBCs or lysed/intact uninfected RBCs (uRBCs) intraperitoneally. After 6 hours, the peritoneal cavity (PEC) fluid was collected. Immune cells isolated from the lavages were subtyped using microscopy and flow cytometry. The levels of 44 immune factors were determined using an ELISA-based test. We observed a difference in immune cell populations and immune factors between mice treated with lysed iRBCs compared to intact uRBCs. Next, we will determine candidate cytokine gene expression, metabolomics of inflammatory cells and extracellular vesicle profile purified from lavage fluid. Then, we will compare these results with results obtained with animals housed at TT following the same protocol in Graphpad Prism™. We expect the IR of mice housed at TT to be significantly different from those at ST due to a difference in immune metabolism. Our results should enable refinement of the malarial mouse model.

727

DYNAMIC MODULATION OF GERMINAL CENTERS BY GUT BACTERIA IMPACT *PLASMODIUM* PARASITE BURDEN

Rabindra K. Mandal[1], Joshua E. Denny[2], Ruth Namazzi[3], Robert O. Opoka[3], Dibyadyuti Datta[1], Chandy C. John[1], Nathan W. Schmidt[1]

[1]*Indiana University, Indianapolis, IN, United States,* [2]*University of Louisville, Louisville, KY, United States,* [3]*Makerere University, Kampala, Uganda*

Gut microbiota educate the immune system in early life to imprint long-term immunological outcomes while also maintaining the capacity to dynamically modulate the local mucosal immune system throughout life. It is unknown if gut microbiota provide signals that dynamically regulate systemic immune responses following an extra-gastrointestinal infection. We show here that gut bacteria provide cues that dynamically modulate germinal center reactions and subsequently the severity of malaria in mice. Importantly, gut bacteria composition was also shown to correlate with the severity of malaria in humans. Whereas antibiotic-induced changes in gut bacteria has often been associated with immunopathology or impairment of host immunity, our data demonstrate that antibiotic-induced changes in gut bacteria populations can enhance humoral immunity to *Plasmodium*. This effect is not universal, but instead depends on the baseline gut bacteria composition. These data provide new insight into the dynamic communications that exist between gut bacteria and the systemic immune response as well as the plasticity of an ongoing humoral immune response.

728

A CONFORMATIONALLY-CONSTRAINED PEPTIDE FROM PVDBP ELICITS ANTIBODIES THAT CROSS-REACT WITH *P. FALCIPARUM* VAR2CSA

Catherine J. Mitran[1], Lauren Higa[1], Michael F. Good[2], Stephanie K. Yanow[1]

[1]*University of Alberta, Edmonton, AB, Canada,* [2]*Institute for Glycomics, Griffith University, Southport, Australia*

Antibodies that cross-react to related antigens in different species of *Plasmodium* can reveal conserved epitopes to pursue as new vaccine candidates. We previously discovered that antibodies to an epitope within subdomain 1 (SD1) of *P. vivax* PvDBP cross-react with the *P. falciparum* placental antigen VAR2CSA and disrupt interactions between VAR2CSA on infected RBCs and the chondroitin sulphate A receptor *in vitro*. Here we explored ways to express the epitope as a vaccine. First, we used a cross-reactive monoclonal antibody, called 3D10, to screen >400 overlapping linear and cyclic peptides made using the CLIPS technology from Pepscan. We identified a discontinuous epitope within SD1 that forms the putative recognition site of 3D10. Based on these findings, one linear peptide and one cyclic peptide were conjugated to carrier proteins and used to immunize BALB/c mice. Immune sera were tested for reactivity to VAR2CSA by ELISA. The linear peptide elicited antibodies to self but failed to cross-react with VAR2CSA. A cyclic peptide of the entire 31 amino acid SD1 sequence elicited antibodies to self with titers >2.0 million and to PvDBP with titres >500,000. However, the antibodies from individual mice differed in their fine specificity toward the immunogen. Antibodies from just over half of the mice also cross-reacted with VAR2CSA, with titers ranging from 1,600 to 6,400. Interestingly, the cross-reactive antibodies had low avidity for the parent protein, PvDBP. Together, these results demonstrate that the epitope in PvDBP that mediates cross-reactivity to VAR2CSA is discontinuous and requires all segments to elicit antibodies with specificity for VAR2CSA. Furthermore, using a cyclic peptide that constrains the conformation of this epitope to its structure in the parent protein, we produced a first-generation vaccine candidate to VAR2CSA based on an epitope from a *P. vivax* antigen.

729

CELLULAR IMMUNE PERTURBATION AND ABERRANT EXPRESSION OF PP38MAPK ON MONOCYTES AND DENDRITIC CELLS IN PEDIATRIC MALARIA

Sidney Ogolla[1], Josselyn Garcia-Perez[2], Conner Jackson[2], Rosemary Rochford[2]

[1]*Kenya Medical Research Institute, KISUMU, Kenya,* [2]*University of Colorado, AMC, Denver, CO, United States*

Plasmodium falciparum malaria causes a complex pattern of immune modulation that primes protection or pathogenesis and is dependent on the dynamic, complex and highly diversified interaction between lymphocytes. To understand this cellular perturbation, we utilized mass cytometry (CyTOF, Cytometry by Time of Flight) a multiparametric analytical platform that enables simultaneous evaluation of 42 markers per cell, providing in depth analysis of cellular heterogeneity. We integrated unsupervised high dimensional analysis of mass cytometry data with clinical data to explore the cellular immune perturbations, followed by targeted flow cytometry to interrogate the functional responses of monocytes and dendritic cells by measuring the phosphorylation of P38 (pP38MAPK) and S6 (pS6). Whole blood samples from children with *Pf* malaria were processed by RBC lysis, fixation and cryopreservation. They were then thawed, barcoded, pooled and stained using a cocktail of antibodies specific to 26 surface markers and analyzed on a Helios mass cytometer. Following stimulation of PBMC's with TLR ligands, we analyzed the expression patterns of pP38 and pS6 on monocytes and dendritic cells by flow cytometry. Mass cytometry data was analyzed using unsupervised approaches visNE and phenograph, a dimension reduction and clustering algorithms, respectively. Flow cytometry data was analyzed by manual gating using Flowjo. Unsupervised data analysis identified 30 distinct cellular clusters with six distinct clusters of naïve $CD4^+$ T cells. We observed significant differences in the frequencies of different B, T cells and monocytes. Most striking was the significant expansion of $CD16^{hi}$, $CD14^{hi}$ monocytes during acute malaria. Analysis of phosphorylation patterns by flow cytometry demonstrated significant phosphorylation of P38 in monocytes and DC cell subsets suggesting the initiation of p38 signal transduction pathway. CyTOF together with unsupervised clustering algorithms comprehensively characterized multiple cell populations with aberrant expression of uniquely expressed markers, thus enabling extensive profiling of cellular subsets.

730

IFN-λ4 GENETIC VARIANTS INFLUENCE CLINICAL MALARIA EPISODES IN A COHORT OF KENYAN CHILDREN

Gabriela Samayoa Reyes[1], Conner Jackson[1], Sidney Ogolla[2], Adeola Obajemu[3], Ludmilla Prokunina-Olsson[3], Arlene Dent[4], Rosemary Rochford[1]

[1]*University of Colorado Anschutz Medical Campus, Aurora, CO, United States,* [2]*Center for global Health research, Kenya Medical research Institute, Kisumu, Kenya,* [3]*Laboratory of Translational Genomics, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National*

Institutes of Health, Bethesda, MD, United States, [4]Center for Global Health and Diseases, Case Western Reserve University, Cleveland, OH, United States

Plasmodium species maintain a complex relationship with the host. Beyond what is known about the roleof type I IFNs, it is possible that type III IFNs, that are active in hepatic cells, could also be playing a role in modulating *Plasmodium* liver stage infection. The production of one type III IFN, IFN-λ4, is controlled by a dinucleotide frameshift variant (rs368234815-dG/TT), carriers of the IFNL4-dG but not the IFNL4-TT alleleare able to produce the IFN-λ4 protein. The effect of this frameshift variation has been studied for hepatitis C virus and for RNA viruses that cause respiratory infections, in both cases the IFNL4-dG allele was associated with unfavorable clinical outcomes. We hypothesized that IFN-λ4 plays a role in modulating innate immune response mounted against *P. falciparum* during liver stage infection. To investigate this hypothesis, we analyzed a cohort of 122 Kenyan children that were followed from birth through 2 years of age with blood samples, *P. falciparum* testing and health questionnaires collected at1520 clinic visits. Blood samples were genotyped for IFNL4-rs368234815. Using the information collected on the health questionnaires from all visits, malaria episodes and respiratory infections were determined and correlated to IFNL4 alleles. The observed IFNL4-dG allele frequency in our study population was 55.3% and genotype frequency – 32.8% dG/dG, 45.1% dG/TT and 22.1% TT/TT, HWE p-value=0.945. We found that carriage of the IFNL4-dG allele, increased the risk and the number of clinical malaria episodes as compared to children with the IFNL4-TT allele. The earlier timing of the first malaria infection was also associated with IFNL4-dG allele, p-value=0.018. We also found that IFNL4-dG allele, increased the risk of respiratory infections as compared to the IFNL4-TT allele, but this difference wasn't significant. Our results are consistent with a longitudinal study of children in Mali (Prokunina-Olsson, submitted) and suggest that the ability to produce IFN-λ4 negatively affects the ability of the host to protect against *P. falciparum.* Future studies are needed to understand the underlying mechanism.

731

DIFFERENTIAL IGG REACTIVITY TO VAR2CSA AND OTHER *PLASMODIUM FALCIPARUM* ANTIGENS AMONG BENINESE PREGNANT WOMEN WITH SICKLE-CELL TRAIT

Mary Lopez-Perez[1], Firmine Viwami[2], Zakaria Seidu[3], Justin Doritchamou[4], Nicaise T. Ndam[3], **Lars Hviid**[1]

[1]*University of Copenhagen, Copenhagen N, Denmark,* [2]*University of Paris, Paris, France,* [3]*University of Ghana, Accra, Ghana,* [4]*Institut de Recherche Clinique du Benin, Cotonou, Benin*

Sickle-cell trait (HbAS) protects against severe *Plasmodium falciparum* malaria. However, placental malaria (PM), which is a major source of mother-offspring mortality and severe morbidity, appears to be an important exception to this general rule. We hypothesized that the reason is that the oxygen tension in the placenta is higher than in the post-capillary venules where infected erythrocytes, including those causing severe malaria in children, normally sequester. Our hypothesis is based on the recent report that HbAS erythrocytes support *in vitro* growth of *P. falciparum* as well as in normal (HbAA) erythrocytes at high oxygen tension, whereas parasite multiplication in HbAS erythrocytes is severely compromised at low oxygen tensions. We measured levels of IgG with specificity for VAR2CSA, which is the PfEMP1-type parasite antigen responsible for sequestration in the placenta, in a cohort of Beninese women with or without sickle-cell trait. We also measured levels of IgG specific for several other *P. falciparum* antigens not restricted to infections with a placental sequestration focus. While levels of VAR2CSA-specific IgG in HbAA and HbAS women were similar, levels to the other antigens were consistently lower in HbAS than in HbAA women. The data thus support our hypothesis regarding the selective lack of HbAS-mediated protection against PM. The results have implications for the understanding of malaria pathogenesis and the evolutionary arms race between *P. falciparum* parasites and acquired host immunity.

732

GUT MICROBIOTA COMPOSITION MODULATES THE MAGNITUDE AND QUALITY OF GERMINAL CENTERS DURING *PLASMODIUM* INFECTIONS

Rafael B. Polidoro[1], Morgan L. Waide[2], Joshua E. Denny[2], Jusrtin Kos[2], David A. Tieri[2], Corey T. Watson[2], Nathan W. Schmidt[1]

[1]*Indiana University School of Medicine, Indianapolis, IN, United States,* [2]*University of Louisville, Louisville, KY, United States*

The gut microbiota has been shown to play a role in both human and rodent *Plasmodium* infections. In mice, gut microbiota composition can modulate the severity of malaria, leading to differences in blood stage parasite burden and the duration of infection. Yet, the mechanism by which gut microbiota impacts the severity of malaria remains unknown. While humoral immunity is critical in mediating clearance of *Plasmodium* blood stage infections, the interplay between gut microbiota, parasite burden, and antibody production is not well characterized. This prompted the hypothesis that mice with lower parasite burden will exhibit better germinal center responses. In support of this hypothesis, there is a gut microbiota-dependent increase in numbers of germinal center B cells and parasite-specific antibody titers that is inversely correlated with parasite burdens. These mice with low parasite burden also demonstrate better maintenance of germinal center structure and a more targeted antibody response. Enhanced humoral immunity during the primary *Plasmodium* infection also impacted memory, as mice with low parasite burden were protected against challenge with a heterologous, lethal *Plasmodium* species. These results provide mechanistic insight on impact of the gut microbiota on extra-gastrointestinal tract germinal center response and will be important in the development optimal treatments for malaria.

733

PRACTICAL EXAMPLE OF MULTIPLE ANTIBODY SCREENING FOR EVALUATION OF MALARIA CONTROL STRATEGIES

Marie-louise Varela[1], **David Koffi**[2], Michael White[3], Mackhtar Niang[1], Babacar Mbengue[4], Fatoumata diene Sarr[1], Andre offianan Toure[2], Ronald Perraut[5]

[1]*Pasteur Institute Dakar, Dakar, Senegal,* [2]*Pasteur Institute Of Cote D'Ivoire, Abidjan, Côte D'Ivoire,* [3]*Pasteur Institute Paris, Paris, France,* [4]*University Cheick Anta Diop, Dakar, Senegal,* [5]*Centre Pasteur Yaounde, Garoua, Cameroon*

Ongoing efforts to fight *Plasmodium falciparum* malaria has reduced malaria in many areas, but new tools are needed to monitor further progress, including indicators of decreasing exposure to parasite infection. Sero-surveillance is considered promising to monitor exposure, transmission and immunity. IgG responses to three antigen biomarkers were evaluated in a retrospective study involving: (i) surveys of 798 asymptomatic villagers from 2 Senegalese endemic settings conducted before 2002 and after the 2013 intensification of control measures, and (ii) in 105 symptomatic individuals from different settings in Côte d'Ivoire. Response to up to eight *P. falciparum* antigens, including recombinant MSP1p9 antigen and $LSA1_{41}$ peptide, were analysed using multiplex technology and responses to whole *P. falciparum* schizont extract (SE, local strain adapted to culture) were measured by ELISA. MSP1p9 and $LSA1_{41}$ IgG responses were shown to be relevant indicators monitoring immune status in the different study sites both from Côte d'Ivoire and Senegal. Between 2002 and 2013, individuals participating in both studies showed a higher decline of sero-positivity in young (< 15 years: range 12% to 50%) than older (> 15 years: no decline to 15%) individuals from Dielmo and Ndiop. A mathematical sero-catalytic model from the complete Dielmo/Ndiop survey was used to reconstruct declining levels of sero-positivity in more detail, demonstrating that anti-SE seroprevalence levels most accurately reflected malaria exposure in the two villages. For standard screening of population immune status at sites envisaging elimination, the use of ELISA-based assays targeting selected antigens can contribute to providing important epidemiologic surveillance data to aid malaria control programmes.

734

EPHRIN B LIGANDS REGULATE CD8+ T CELL FUNCTION IN EXPERIMENTAL CEREBRAL MALARIA

Adesola C. Olatunde, Mellina Srey, Jared Andersen, Patrice Mimche, Spencer Seely, Nicole Stokes, Tracey J. Lamb

University of Utah, Salt Lake City, UT, United States

Cerebral malaria (CM) is a major fatal complication associated with *Plasmodium falciparum* infection in humans. Induction of experimental cerebral malaria (ECM) in C57BL/6 mice upon infection with *Plasmodium berghei* ANKA (*Pb*A) recapitulates some of the clinical symptoms of CM in humans. This model requires antigen-specific $CD8^+$ T cells for disease pathology. However, the molecules that govern T cell migration and trafficking to the brain is incompletely understood. In this study, the role of T cell-expressed EphrinB (EfnB) ligands that bind the Eph B receptor tyrosine kinase subfamily was examined during ECM. Using the *Pb*A model of ECM, we observed that activated splenic $CD8^+$ T cells highly express EfnB ligands, particularly in conjunction with high expression of IFN-γ and TNF-α. Based on published data, we hypothesized that EfnB ligands regulate T cell chemotaxis and recruitment to the brain during ECM. Consistent with this hypothesis more than 90% $CD8^+$ T cells reactive to the *P. berghei* ANKA GAP50 peptide by tetramer straining expressed EfnB in the brain. Selective deletion of EfnB1 and EfnB2 on T cells ($CD4^{cre+}EfnB1/B2^{fl/fl}$ mice) led to a decrease in the number of activated antigen-specific $CD8^+$ T cells in the brain of EfnB1/B2 deficient mice. To define genes that were differentially expressed by $CD8^+EfnB^+$, $CD8^+EfnB^-$ and naïve $CD8^+$ T cells, cells were FACs sorted into three populations and analysis by RNA-sequencing. Among the genes that were upregulated in $CD8^+EphB^+$ cells were genes associated with T cell chemotaxis. Taken together, our data suggest a potential role for EfnB ligands in T cell trafficking to the brain during ECM.

735

MODELING THE DROP-OFF IN THE PROTECTIVE EFFICACY OF SEASONAL MALARIA CHEMOPREVENTION FROM CLINICAL TRIALS TO PROGRAMMATIC IMPLEMENTATION IN BURKINA FASO

Sebastian Rodriguez[1], Cheick Saïd Compaore[2], Boulaye Dao[2], Beatriz Galatas[3], Abdisalan Noor[3], Jaline Gerardin[1], Noelle Samia[1]

[1]*Northwestern University, Evanston, IL, United States,* [2]*National Program for the Fight Against Malaria, Ouagadougou, Burkina Faso,* [3]*World Health Organization, Geneva, Switzerland*

Seasonal malaria chemoprevention (SMC) was first recommended by the WHO in 2012 as a preventive therapy for children in the Sahel region between 3-59 months of age. The foremost recommended treatment, 1 dose of sulfadoxine/pyrimethamine (SP) combined with a 3-day course of amodiaquine (AQ), is administered during the peak malaria transmission period once a month for up to 4 months. The goal of SMC campaigns is universal coverage of children in the desired age-group to achieve a prophylactic effect among eligible children during the rainy season, corresponding to the period of greatest vulnerability to malaria. Meta-analysis of clinical trials analyzing SMC efficacy on clinical malaria have found a 75% decrease in disease incidence in the treatment group relative to the control group. However, a drop-off of about 15% in protective efficacy has been observed in Mali and Burkina Faso when moving from the clinical setting to a programmatic setting, potentially due to seasonal or operational limitations affecting efficacy itself, coverage, or adherence to the full treatment course. By utilizing routine case data from health districts in Burkina Faso, we quantify the protective efficacy of SMC and identify covariates that drive the drop-off between clinical and programmatic efficacy of SMC. Preliminary results on yearly referenced data show an average incidence reduction ratio of 0.20 on uncomplicated malaria, meaning roughly 20% of cases are averted when districts implement SMC. We employ geostatistical models in a Bayesian framework to account for the spatial variation in SMC efficacy across districts. These include conditional or simultaneous autoregressive (CAR and SAR) models that leverage the spatial heterogeneity of the region and time-dependent structure of the data. Using confirmed malaria cases, malaria hospital admissions, all-cause hospital admissions, local climate variables, and care-seeking indicators, we explain the differences in observed SMC impact between health districts that drive the drop-off in protective efficacy. The results of this work can inform district level programs in increasing the effectiveness of SMC.

736

ESTIMATING THE POTENTIAL EFFECTIVENESS OF WIDE-SCALE IMPLEMENTATION OF INTERMITTENT PREVENTIVE THERAPY IN INFANTS IN SOUTHERN NIGERIA

Manuela Runge[1], Ifeoma D. Ozodiegwu[1], Brie Falkard[2], Jaline Gerardiin[1]

[1]*Northwestern University, Chicago, IL, United States,* [2]*Bill & Melinda Gates Foundation, Seattle, WA, United States*

Nigeria accounts for 23% of malaria deaths globally with the highest burden in children under the age of five. Intermittent preventive therapy in infants (IPTi) aims to reduce clinical malaria episodes and deaths in infants and is recommended in moderate to high, non-seasonal, transmission areas. Around 374 local government areas (LGAs) in Southern Nigeria have been identified as eligible for IPTi. However, the impact of IPTi is highly uncertain and will depend on the achievable coverage. This study uses a scalable modeling approach to provide estimates of IPTi impact per LGA in Southern Nigeria. A literature search was conducted to identify clinical trials and extract IPTi's protective efficacy on clinical cases, prevalence, anemia, and malaria-attributed deaths. Malaria burden predictions for 2020-2025 were obtained from a mathematical transmission model, previously calibrated for each LGA in Nigeria including varying case management and insecticide treated bed net coverage scenarios. Since the implementation of IPTi is likely to be paired with the Expanded Immunization Program, vaccine coverage from the 2018 demographic health surveys were used to inform the average IPTi coverage. The IPTi coverage adjusted with an increase of 20%, ranged from 20 to 100% and was assumed to stay constant. Across the southern LGAs, the additional impact of IPTi among infants was estimated at around 18%, 29% and 6% reduction in incidence, prevalence, and mortality respectively over the five-year period, with the highest number of total cases and death averted in highly populated areas. Combining IPTi protective efficacy, LGA-specific estimates of coverage and future malaria burden predictions, our estimations of malaria-related outcomes account for differences in transmission intensity and intervention impact across Southern Nigeria. The results suggest that while relative reductions in mortality are low, the total number of infant lives saved can be considerable and supports the implementation of IPTi given the protective efficacy as seen in clinical trials and high coverage. To obtain more up to date predictions more recent data would be beneficial.

737

SUPPORTING STRATEGIC PLANNING WITH MALARIA MODELLING IN MOZAMBIQUE

Munir Winkel[1], Eva de Carvalho[2], Ana Rita Chico[3], James Colborn[3], Bradley Didier[3], Dominic Lucero[3], Mathe Guidon[2], Arantxa Roca-Feltrer[4], Rose Zulliger[5], Baltazar Candrinho[2], Emilie Pothin[1]

[1]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [2]*National Malaria Control Programme, Maputo, Mozambique,* [3]*Clinton Health Access Initiative, Boston, MA, United States,* [4]*Malaria Consortium, Maputo, Mozambique,* [5]*US Centers for Disease Control and Prevention, Atlanta, GA, United States*

In line with the World Health Organization's High Burden to High Impact initiative, countries are revising their national strategies by incorporating evidence-based approaches, whilst donors are using evidence to guide funding decisions. In 2020, Mozambique's National Malaria Control Program updated its national stratification plan (NSP) and adopted

model-based approaches to inform decisions for implementing case management and vector control interventions in its districts. The impact of case management, seasonal malaria chemotherapy (SMC), long-lasting insecticide-treated pyrethroid nets (LLINs), piperonyl butoxide (PBO) nets, and indoor residual spraying (IRS) were simulated using OpenMalaria, a mathematical model that mimics the dynamics of malaria transmission. Model parameters for vectors were based on studies measuring mosquito mortality in the presence of interventions. The model was calibrated to malaria prevalence from the malaria indicators surveys from 2007, 2011, 2015, and 2018; and estimated per district by the Malaria Atlas Project. Mozambique's current stratification includes a mix of LLIN, IRS, LLIN + IRS, and PBO/next-gen nets covering 3, 11, 23, and 63 percent of the population, respectively. Local empirical evidence and operational feasibility informed the prioritization for LLIN, IRS, and PBO districts. Modelling suggested that the current NSP would lead to an 8% reduction in cases in children under five when compared to deploying only pyrethroid LLINs. Additionally, it helped to prioritize where SMC could be implemented. Modelling is a useful tool to simulate the impact of interventions, although it is subject to various sources of uncertainty. Several assumptions are made to represent the malaria dynamics, especially with limited availability of data on intervention effectiveness, poor quality of geographic-specific estimates of historical malaria trends and intervention uptake. Mathematical models helped guide strategic decisions by supporting evidence-based prioritization of interventions during the NSP and the 2020-2022 Global Fund application.

738

ESTIMATING MULTIPLICITY OF INFECTION AND ALLELE FREQUENCIES AND PREVALENCES ACCOUNTING FOR MISSING DATA

Meraj Hashemi, Kristan A. Schneider

Hochschule Mittweida, Mittweida, Germany

Multiplicity of infection (MOI) refers to the number of super-infections due to the occurrence of multiple infectious contacts. Accurate estimates of MOI based on SNP or microsatellite data are highly desirable, as it is revealing clinical, genetic, and epidemiological purposes. Due to limitations and difficulties of molecular methods for sequencing SNPs or calling STRs, missing data or undetected alleles are common. Although, estimating MOI and allele (or lineage) frequencies/prevalences are recognized to be fundamental in malaria genetic studies, unobserved and missing genetic/molecular information is not properly accounted for by current methods. This potentially biases results and reduces the confidence of estimates. Here, we develop a statistical model to estimate MOI and allele frequencies and prevalences from molecular data containing missing molecular information. The model assumes that alleles/lineages present within an infection will be detected in a blood sample independently of each other, potentially leading to samples with completely missing information. We apply the EM algorithm to derive maximum-likelihood estimates (MLE) of the MOI distribution, allele/lineage-frequency spectrum. The distinct feature of this method is that it incorporates patient blood samples with completely missing information. The method has desirable analytical (asymptotic) properties. Furthermore, as shown by a systematic numerical study, the method performs well for realistic sample sizes. As an example, the method is applied to a data set previously collected in Asembo Bay, Kenia. An easy to use implementation of the method to estimate allele frequency spectra at a single SNP or microsatellite locus alongside MOI in R is provided.

739

MALARIA TEMPORAL VARIATION AND MODELING USING TIME-SERIES IN SUSSUNDENGA DISTRICT, MOZAMBIQUE

João L. Ferrão[1], Dominique Earland[2], Anísio Novela[3], Alberto Tungadza[4], Kelly M. Searle[2]

[1]*Instituto Superior de Ciências de Educação, Beira, Mozambique,* [2]*University of Minnesota, Minneapolis, MN, United States,* [3]*Direcção Distrital de Saúde de Sussundenga, Manica, Sussundenga, Mozambique,* [4]*Universidade Católica de Moçambique em Beira, Beira, Mozambique*

Malaria is one of the leading causes of morbidity and mortality in Mozambique with the 5th highest prevalence in the world, with little progress in malaria control over the past 20 years. Sussundenga district in Manica Province has documented high *Plasmodium falciparum* incidence at the local rural health center (RHC). Sussundenga is a rural district located along the Mozambique, Zimbabwe border. *P. falciparum* transmission in this area is unique as there are differing control policies on each side of the international border. The study objective was to analyze the *P. falciparum* temporal variation and model its pattern in Sussundenga district, Mozambique. Data from weekly epidemiological bulletins (BES) were collected from 2015 to 2019, which records confirmed *P. falciparum* cases from health facilities. These data categorize confirmed cases into two age groups: under 5 years and 5-years and older. *P. falciparum* incidence and temporal variation were calculated. Temporal clusters were identified using dendrograms. A time-series analysis was carried out. For temporal modeling a Box-Jenkins method was used applying an autoregressive integrated moving average (ARIMA). Over the study period, 372,498 cases of *P. falciparum* were recorded in Sussundenga, 177,957 from under 5 years (47.5 %) and 194,541 (52.2 %) from 5 years and older. There were weekly and yearly variations in incidence overall ($p < 0.001$). There was a decreasing trend in cases for those under 5 years while there was a slight increase in those 5 and older. For those under 5, week 2 of the year had the highest number of cases (1170 sd 34) while week 35 had the fewest (354 sd 17.8). For those 5-years and older, cases also peak at week 2 (1295 sd 245) with week 31 having the fewest cases (341 sd 193.5). The findings indicate that cases are decreasing in those under 5 years and are increasing slightly in those 5 years and over. *The P. falciparum* case occurrence presents a weekly temporal pattern peaking during the wet season. Based on the temporal distribution, more efficient strategies that target this seasonality can be implemented to reduce the overall malaria burden in Sussundenga District, and regionally.

740

PREDICTING THE PUBLIC HEALTH IMPACT OF A MALARIA TRANSMISSION-BLOCKING VACCINE

Joseph D. Challenger[1], Daniela Olivera Mesa[1], Dari F. Da[2], Serge R. Yerbanga[2], Thierry Lefevre[3], Anna Cohuet[3], Thomas S. Churcher[1]

[1]*Imperial College London, London, United Kingdom,* [2]*Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso, Burkina Faso,* [3]*University of Montpellier, Montpellier, France*

There is growing interest in transmission blocking vaccines (TBVs), which interrupt malaria transmission from humans to mosquitoes. Although such vaccines are being tested in early clinical trials, the activity of a TBV is commonly evaluated using membrane feeding assays. Data on naturally infected mosquitoes collected in Burkina Faso is used to translate a vaccine's laboratory-estimated activity into an estimated activity in the field. An established model of malaria transmission is utilised to predict a TBV's public health impact. Designing a vaccination campaign for a TBV requires an understanding of which age groups contribute the most to transmission. In our modelling work, for both a low- and high-transmission setting, we estimate that vaccinating children between 8 and 10 years old would have double the impact of vaccinating the same number of 2- to 4-year-olds or 15- to 18-year-olds. The benefits of vaccination are distributed across the population, averting the greatest number of cases in younger children. The addition of a TBV to existing control interventions

such as insecticide-treated nets (ITNs) could have a substantial impact against malaria, particularly in settings where the impact of ITNs is diminished by insecticide resistance.

741

MODEL INFORMED TARGET PRODUCT PROFILES OF LONG ACTING INJECTABLES FOR USE AS SEASONAL MALARIA PREVENTION

Lydia Burgert[1], Mirjam Laager[1], Monica Golumbeanu[1], Jörg J. Möhrle[2], Melissa A. Penny[1]

[1]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [2]*Medicines for Malaria Venture, Geneva, Switzerland*

Seasonal malaria chemoprevention (SMC) has alleviated a substantial amount of malaria burden, its efficacy to reduce malaria incidence being extensively demonstrated in several clinical trials. in 2018 alone over 19 million children aged between 3-59 months received monthly Sulfadoxine-Pyrimethamine+Amodiaquine (SP-AQ) during seasonal malaria transmission and benefited from its protective effects. However, these gains in global health are threatened by the spread of resistance against SP, operational complications in delivery, and incomplete adherence to the regimen, both within one treatment course and over the whole season. Long acting injectables (LAIs), in form of small molecule drugs or monoclonal antibodies, could provide an alternative prevention tool by simplifying the deployment and reducing the risk of resistance. Here, we quantitatively assessed and redefined existing target product profiles (TPPs) for LAIs. We investigated the optimal implementation setting, regimen, protective efficacy of LAIs using simulation modelling with an established individual-based modelling platform, OpenMalaria. After calibration of the simulated protective efficacy of SMC with SP-AQ to existing clinical trial data, we assessed the conditions of non-inferiority of LAI to SMC and explored trade-offs between tool properties and operational constraints. Sensitivity analysis through a Gaussian-process emulator identified half-life and deployment coverage as the main drivers of LAI effectiveness in reducing malaria incidence across a wide range of transmission intensities. Additionally, the establishment of non-inferiority of LAIs to SMC was highly dependent on the transmission intensity and presumed resistance against SP. The TPP of LAI as an effective replacement to an existing SMC program was found to be highly dependent on operational factors of the new LAI and of existing SMC programs in terms of deployment coverage and adherence. Consequently, the importance of operational factors alongside product profiles of LAI should be carefully evaluated, as well as the malaria epidemiology of likely implementation settings.

742

MACHINE LEARNING-BASED APPROACHES TO PARAMETERIZING INDIVIDUAL-BASED MODELS OF MALARIA DYNAMICS

Theresa Reiker[1], Ewan Cameron[2], Sarah Filippi[3], Melissa Penny[1]

[1]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [2]*University of Oxford, Oxford, United Kingdom,* [3]*Imperial College, London, United Kingdom*

Over the last decades individual-based models have taken a valuable role in the global battle against many infectious diseases, from understanding their dynamics to assessing interventions and predicting epidemic trajectories. However, model complexity makes calibration to field data difficult. The parameterization of individual-based models is a multidimensional, often multi-objective optimization problem, where evaluation of the true (simulator) function is costly both in terms of time and computational resources. Commonly employed MCMC-based solutions generally do not attempt to fit to multiple objectives simultaneously and often do not harvest the increased availability of computational resources in recent years. It is thus often uncertain whether global or local minima were reached. Here, we revisit the parameterization of OpenMalaria, an established malaria modelling platform. OpenMalaria is calibrated to a range of data sources representing multiple epidemiological outcomes, from age-specific prevalence and incidence patterns to age-specific mortality rates and hospitalisation rates to ensure that the model realistically represents malaria transmission. We propose a new approach to calibrate complex models via a Bayesian Optimization framework with different machine learning emulator functions thus solving this optimization problem which contains 23-dimensional parameter input space with 10 parallel fitting objectives. We show that our approach outperforms previous MCMC-based calibration attempts, both in terms of runtime and final goodness of fit. The implications of our results are threefold: The new calibration paves the way to more accurate models and model predictions. Secondly, the emulator approach also allows for variable selection as well as easy sensitivity and importance analyses, providing additional insights into these multidimensional spaces. Lastly, the algorithm provides fast solutions to broader multidimensional optimization questions in disease modelling, including but not limited to calibration.

743

UNDERSTANDING SPATIO-TEMPORAL MOBILITY FOR MALARIA RISK AND TRANSMISSION USING A MULTI-AGENT SIMULATION MODEL

Kathleen Stewart[1], Yao Li[2], Nay Y. Linn[3], Zaw Lin[4], Tint Wai[3], Kay Thwe Han[5], Zay Yar Han[6], Elizabeth Turner[7], Alyssa Platt[7], Poe. P. Aung[7], Zaw W. Thein[7], Thura Htay[7], Christopher V. Plowe[7], Myaing N. Nyunt[7]

[1]*University of Maryland, College Park, MD, United States,* [2]*Yao Li, College Park, MD, United States,* [3]*National Malaria Control Program, Myanmar Ministry of Health and Sports, Nay Pyi Taw, Myanmar,* [4]*National Malaria Control Program, Myanmar Ministry of Health and Sports, Nay Pyi Taw, Mozambique,* [5]*Department of Medical Research, Myanmar Ministry of Health and Sports,, Yangon, Myanmar,* [6]*Department of Medical Research, Myanmar Ministry of Health and Sports, Yangon, Myanmar,* [7]*Duke Global Health Institute, Duke University, Durham, NC, United States*

Human mobility paves a pathway for the movement of malaria parasites, and plays a critical role in understanding malaria risk, and the origin ("sources") and destination ("sinks") of malaria transmission. Human movement across areas of differing transmission intensity increases the heterogeneity of malaria risk which, in turn, elevates the transmission intensity for a given population. In this study, the relationship between human mobility and *Plasmodium falciparum* malaria risk was assessed in 37 targeted villages in Singu Township, Mandalay Region, one of six regions slated for subnational malaria elimination in Myanmar. Extensive data on occupation, time, distance, duration, modes of travel, and other related information were collected using a questionnaire. Spatio-temporal patterns for occupation-related travel were constructed from February to August 2018, using a multi-agent-based model. Individual malaria status determined using ultrasensitive polymerase reaction (usPCR) methods, and village-level malaria prevalence were estimated. A malaria risk surface for the area, i.e., the probability of 1% *P. falciparum* prevalence by usPCR across the township, was estimated using remotely sensed environmental variables and a maximum entropy (Maxent) model. Simulated spatio-temporal mobility patterns for different occupations were compared with the mapped risk surface to evaluate where daily movements and higher risk of malaria overlap. A quantitative risk indicator was constructed for each agent to model how risk varies across different occupation groups. The study findings showed that mobility associated with loggers and plantation workers had an average risk index value about three times larger than the values for farmers, and one and a half times larger than for miners. Morning mobility patterns for *P.f.* positive cases overlapped most frequently with higher malaria risk areas. These findings indicate that daily mobility should be considered as an important component in malaria control and elimination efforts.

745

UTILIZING SPATIAL MAPS OF ANTIMALARIAL PARTNER DRUG RESISTANCE TO IDENTIFY PRIORITY REGIONS FOR SURVEILLANCE

Hanna Ehrlich, Daniel M. Weinberger, Joshua L. Warren, Sunil Parikh

Yale University, New Haven, CT, United States

The treatment of *Plasmodium falciparum* in sub-Saharan Africa relies on artemisinin-based combination therapies (ACTs). Molecular markers provide rapid information on landscapes of resistance to ACTs and can protect the efficacy of artemisinin by informing regional selections of longer-acting partner drugs. We hypothesized that molecular markers demonstrate spatial patterns that, together with predicted areas of uncertainty, could be leveraged to locate high-priority sites for resistance surveillance. Through a systematic search, we identified 501 geo-located surveys assessing well-known molecular markers associated with partner drug susceptibility– *pfmdr1* N86Y, Y184F, D1246Y and/or *pfcrt* K76T– in sub-Saharan Africa from 2004-2018, the largest database to date. We found evidence of spatial autocorrelation in survey data for all alleles (*Moran's I*= 0.30–0.55, $p<0.0001$). Using hierarchical Bayesian spatial modeling with environmental and epidemiological covariates, we predicted the prevalence of markers in 608 first-level administrative divisions (ADs) in sub-Saharan Africa from 2004-2009 and 2010-2018, corresponding to the respective uptake and widespread use of ACTs. The maps showed continent-wide selections of *pfmdr1* N86 and D1246, which were significantly associated with changes in ACT coverage and drug policy. The maps also support prior localized reports of re-expansion of *pfcrt* wild-type alleles, with posterior prevalence of mutations decreasing in 96% of ADs from an overall mean of 0.76 to 0.43 over the two time periods. Finally, because current surveillance efforts often fail to capture spatial-temporal heterogeneity in the distribution of resistance, we identified ADs with the highest standardized prevalence and uncertainty combined estimates for prioritized surveillance. Within selected ADs, we determined optimal placement of future sentinel sites that accounted for operational and design constraints. Our work will assist in efforts to sustain the use of ACTs as first-line therapies and improve disease surveillance strategies more broadly.

746

TREATMENT-SEEKING BEHAVIOR FOR FEVER IN KINSHASA PROVINCE, DRC: IMPLICATIONS FOR MALARIA CASE MANAGEMENT

Kristin E. Banek[1], Melchior Kashamuka Mwandagalirwa[2], Alpha Oumar Diallo[1], Georges Emo Mahilu[2], Joseph A. Bala[2], Georges Kihuma[2], Joseph Atibu[2], Marthe Nkalani[2], Tommy Nseka Mambulu[2], Jonathan J. Juliano[1], Jonathan B. Parr[1], Steve R. Meshnick[1], Antoinette Kitoto Tshefu[2]

[1]*University of North Carolina-Chapel Hill, Chapel Hill, NC, United States,* [2]*Ecole de Santé Publique, Faculté de Médecine, University of Kinshasa, Kinshasa, Democratic Republic of the Congo*

The Democratic Republic of the Congo (DRC) has one of the highest burdens of malaria in Africa. One of the cornerstones of malaria control in the DRC is confirmatory diagnosis and effective treatment with artemisinin-based combination therapy (ACT). Despite the adoption of effective case management strategies, implementation challenges, such as access and acceptance of diagnostic testing and treatments, still exist. This study quantifies the level of treatment-seeking behavior and access to confirmatory diagnosis and treatment with ACT in participants with self-reported fever in the context of an ongoing longitudinal study in Kinshasa Province, DRC. Data were collected during an active surveillance household survey in March 2020. Participants were asked about recent fever, treatment-seeking practices, whether they received a malaria test, and which treatment was received. Preliminary analyses describe the proportion of participants seeking and receiving malaria testing and treatment. Additional analyses are planned to explore the potential factors associated with treatment-seeking and adherence to treatment guidelines. Of the 892 active participants, 128 (14.4%) reported fever in the week preceding the interview, and 82% (105/128) sought care. The majority (93%) sought treatment the same or the next day after the fever began. However, two-thirds of these self-treated at home and only 20% (21/105) sought care at the health facility and received a confirmatory blood test for malaria. All 21 participants visiting the health facility received an antimalarial, 85% (18/21) had a positive malaria test, and 67% (14/21) reported receiving an ACT. While it is expected study participants study seek care early, these findings highlight that the majority of participants are self-treating at home first without a confirmatory test. Further analysis will provide essential information necessary to improve the implementation of malaria treatment guidelines so that participants seek treatment promptly at the health facilities so that they can receive confirmatory diagnosis and the correct treatment for malaria.

747

MALARIA PREVALENCE AND CARE SEEKING BEHAVIORS PRIOR TO A PILOT EXPANDING MALARIA COMMUNITY CASE MANAGEMENT TO OLDER AGES IN FARAFANGANA, MADAGASCAR, 2019

Dean Sayre[1], Laura Steinhardt[2], Judickaelle Irinantenaina[3], Catherine Dentinger[4], Laurent Kapesa[4], Joss Razafindrakoto[5], Agathe Legrand[3], Nicole Prada[3], Annett Cotte[1], Shelby Cash[1], Julie Gutman[1], Lauren Lewis[1], Tsinjo Rasoanaivo[3], Reziky Mangahasimbola[3], Andres Garchitorena[3], Aina Harimanana[3]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Centers for Disease Control and Prevention, Abuja, Nigeria,* [3]*Institut Pasteur de Madagascar, Antananarivo, Madagascar,* [4]*Presidents Malaria Initiative, Antananarivo, Madagascar,* [5]*USAID, Antananarivo, Madagascar*

Integrated community case management of malaria, pneumonia, and diarrhea is a proven intervention to reduce mortality in children less than five years old, and there is growing interest to expand malaria community case management (mCCM) to older individuals. Prior to a two-year cluster-randomized trial of the expansion of mCCM to all ages, we conducted a cluster-sample household survey in the Farafangana district of Madagascar to assess healthcare seeking behaviors and childhood malaria prevalence (among children < 15 years of age) by rapid diagnostic test (RDT). The study was performed in 30 different health facility catchment zones, with two study enumeration areas randomly chosen from each. Within each enumeration area, 28 households were randomly chosen for study inclusion. Weighted population estimates and Rao-Scott chi-squared tests were used to adjust for study design. Of 8,050 individuals in 1,458 households surveyed, 459 (6.7%) reported illness in the previous two weeks; the majority of these (375/459; 82.3%) reported fever as part of this illness. Of those reporting fever, 28.7% (112/375) sought care; this did not differ by participant age ($p = 0.66$). Most participants seeking care for fever visited public health facilities (HFs) (48/112, 46.8%), or community healthcare volunteers (CHVs) (40/112, 31.0%). Of those presenting with fever at HFs and CHVs, 87.0% and 71.0%, respectively, reported being tested for malaria. Malaria prevalence among children < 15 years old according to the survey RDT was 25.4% (CI: 19.8-31.1%, 761/3317). Prevalence increased with age: 7.3% in < 2-year-olds; 19.6% in 2-4-year-olds; 28.6% in 5-9-year-olds; and 36.2% in 10-14-year-olds ($p < 0.001$). While care-seeking for febrile illness was uncommon, rates of malaria testing for those who sought care at a publicly supported healthcare provider were high. Expanding community case management to older individuals, along with campaigns to encourage care-seeking, could potentially reduce the burden of malaria in this area by increasing appropriate testing and treatment.

748

COMMUNITY ENGAGEMENT TO STRENGTHEN MALARIA ELIMINATION IN FOUR PROVINCES IN VIETNAM

Hong Hoa Nguyen, Trung Nguyen, Tung Vu, Le Hoa Nguyen, Tuan Nguyen

Population Services International Vietnam, Hanoi, Viet Nam

Vietnam has made significant progress in the last decade towards its commitment to eliminate malaria by 2030, however, progress has recently stalled. Forest-goers are at the highest risk of malaria in Vietnam. Community engagement (CE) is a proven strategy to empower communities to identify health problems and identify locally-owned solutions. In 2019, we implemented 30 community dialogues and 114 screening events with communities in Khanh Hoa, Gia Lai, Dak Lak and Binh Phuoc provinces to promote malaria case management by pharmacists and trained informal private providers and strengthen community members' malaria knowledge. Malaria tests were conducted by providers from the local communities.Between Oct and Dec 2019, we conducted exit interviews with 160 forest-goers randomly selected from 10 dialogues and 8 screening events. Data was captured on malaria knowledge and satisfaction with the events. Routine data from monthly program reports were extracted to assess the number of participants, tests performed and cases detected. CE events reached 7,593 people in 144 communities. In total, 5,193 malaria diagnostic tests were performed and 29 malaria cases identified. 24/29 cases were male and aged 15 years and above (the most at-risk population for malaria in Vietnam). Among exit interview respondents, 70% reported greater confidence in their knowledge of malaria and 80% found the information shared useful. Over 90% of respondents were satisfied with the attitude and skills of the health care providers at the events, and 98% of respondents reported increased confidence in being able to seek prompt treatment from providers.In 2018, Vietnam identified 4,811 cases from 1,926,252 tests performed (0.25% case detection). Our CE events achieved a case detection level of 0.56% and represented 3.3% of all cases detected by the project in Vietnam in 2019. Targeted community engagement events can be used to test, treat and track malaria cases while increasing malaria knowledge and building spaces for ongoing provider-community dialogues for behavior change to support malaria elimination.

749

DIFFERENTIAL MALARIA PREVENTION BEHAVIORS AND CARE SEEKING PRACTICES BETWEEN WORKSITE MIGRANT WORKERS AND VILLAGERS IN THE MALARIA-AT-RISK AREAS IN MYANMAR

Ye Kyaw Aung, May Me Thet, Si Thu Thein

Population Services International, Yangon, Myanmar

Migrant populations are at an increased risk of exposure to malaria due to their nature of work and seasonal migration. This study aimed to differentiate malaria prevention behaviors and care seeking practices among worksite migrant workers and villagers in the malaria-at-risk areas of the Eastern Myanmar close to the Chinese border. A mixed-method study was conducted during March 2019. The malaria-at-risk worksites in the targeted four townships, and villages located at the nearest to these worksites were approached. The key stakeholders, such as worksite managers and village leaders, were interviewed. A total of 23 worksites, which employed 880 migrants and 447 locals, and 20 villages, which were homes for 621 migrants and 9731 locals, was successfully interviewed. Regarding malaria prevention behaviors, sleeping under bed net was common among both worksites (74%) and villages (85%). In contrast, Long-lasting-insecticidal-nets (LLIN) usage was much lower among the worksites than villages (39% vs 80%). Regarding care seeking practices, self-medication was a popular choice for both workers and villagers owing to easy availability of Western medicine. In contrast, local-belief-driven traditional practices were more common among villagers. On occasions when fever was not relieved, both would seek health care from rural health centers, private clinics, or public hospital. As for barriers, villagers mostly cited language barriers, which often led to misunderstanding between health providers and them. In contrast, most of the worksites cited logistic issues as they were in remote areas with devastated road conditions and the routes to formal health facilities were not secure due to frequent armed conflicts. This study demonstrated that site-workers and villagers had different malaria prevention behaviors and care seeking practices even though they resided in the same geographic area. Hence, it is important to recognize such differences for more effective intervention approaches.

750

IMPACT OF PRIVATE HEALTH SECTOR ENGAGEMENT INTERVENTIONS ON PROVIDER QUALITY OF MALARIA CASE MANAGEMENT IN CAMBODIA, LAO PDR, MYANMAR AND VIETNAM

Kemi Tesfazghi[1], Sochea Phok[2], Saysana Phanalasy[1], Si Thu Thein[3], Hong Hoa Nguyen[4], Stephen Poyer[5]

[1]*Population Services International Laos, Vientiane, Lao People's Democratic Republic,* [2]*Population Services International Cambodia, Phnom Penh, Cambodia,* [3]*Population Services International Myanmar, Yangon, Myanmar,* [4]*Population Services International Vietnam, Hanoi, Viet Nam,* [5]*Population Services International, Washington DC, DC, United States*

Sustained private sector engagement is necessary to achieve malaria elimination in the Greater Mekong Subregion (GMS), where 40-78% of the population first seek care for fever in the private sector. In 2015, representative outlet surveys identified low availability of malaria diagnostic testing and access to first-line treatment in the private sector across the GMS. From 2015 to 2019 PSI implemented interventions in four countries to increase the coverage of quality malaria case management among private health providers. Between August and December 2019, we conducted representative cross sectional mystery client (MC) surveys among private outlets in Cambodia, Lao PDR, Myanmar and Vietnam to assess provider quality of care. Confirmed RDT-negative volunteers with no reported fever in the past four weeks were recruited to assess provider adherence to national treatment algorithms for test-negative patients by presenting with reported recent malaria symptoms. We captured information on testing availability, diagnostic services, medicines prescribed/sold and provider counseling. In total 1304 eligible client visits were made to participating outlets in the four countries. Unprompted malaria testing occurred in 43% (Myanmar) to 77% (Cambodia) of client visits. When prompted, testing levels increased to over 95% in Cambodia and Lao PDR, but were lower in Myanmar (51%) and Vietnam (69%). Provider provision of antimalarials following a negative RDT result was rare (0-1.5%); Myanmar had the highest number of non-compliant cases (4). Provider adherence to critical RDT steps was heterogeneous by country: gloves were worn in 26%-60% of visits, and 38% providers in Myanmar and 16% in Vietnam waited less than the recommended time before reading the RDT. These comparable survey results reveal varied levels of diagnostic testing and adherence to RDT processes across countries (and provider cadres, not shown) despite high fidelity intervention implementation in recent years. The results provide an objective complement to routine program activities that assess provider quality and can provide valuable information for program improvement.

751

INSIGHTS ON FOREST-GOER HEALTH SEEKING JOURNEYS FOR FEBRILE ILLNESS IN CAMBODIA AND VIETNAM USING RESPONDENT-DRIVEN SAMPLING

Mahesh Paudel[1], Sochea Phok[2], Hong Hoa Nguyen[3], Stephen Poyer[1], Kemi Tesfazghi[4]

[1]*Population Services International, Washington DC, DC, United States,* [2]*Population Services International Cambodia, Phnom Penh, Cambodia,* [3]*Population Services International Vietnam, Hanoi, Viet Nam,* [4]*Population Services International Laos, Vientiane, Lao People's Democratic Republic*

Substantial progress has been made towards malaria elimination in the Greater Mekong Subregion. However, malaria continues to disproportionately affect forest workers and migrants. Due to forest-goers' hard-to-reach and often mobile status, novel data collection methods are required to gain behavioral insights from this group to inform appropriate malaria elimination interventions.In 2019 we conducted a population-based survey of forest goers in Cambodia and Vietnam using respondent-driven sampling (RDS) to measure malaria related behaviors. Forest-goers were defined as people who live within 15km of the forest and visit the forest at least 1 night a week or 4 nights a month. A total of 648 (Vietnam) and 675 (Cambodia) forest-goers were recruited from 15 and 21 initial seeds. Data were analyzed using the RDS model in Stata 15.All participants in Cambodia and 66% in Vietnam reported an episode of fever in the past 30 days. Among these respondents, 75% (Cambodia) and 63% (Vietnam) sought treatment outside the home, though levels of prompt care seeking were suboptimal (28% Cambodia; 33% Vietnam). Recourse to a private health facility was more common in Cambodia than Vietnam (37% vs. 19%), while public sector treatment seeking was more common in Vietnam (46% vs 18%). A quarter of respondents sought care from community-based providers in Cambodia (24%) while this was not cited as a source of care in Vietnam. The top three reasons cited for forest-goers' choice of treatment source in Vietnam were proximity (88%), cost (73%) and perceived service availability (49%). In Cambodia, trust in providers (64%), cost (62%) and proximity (56%) was the top three reasons for choosing a provider. Forest going is a major risk factor for malaria in Cambodia and Vietnam, however economic pressures compel people to undertake this activity. Programs can use RDS as a powerful tool to gather insights into forest-goers' behaviors and inform the design and targeting of malaria elimination interventions. In Cambodia these results are being used to contribute to national behavior change guidelines, design tools and refine interventions.

752

STAKEHOLDER ENGAGEMENT FOR PREDICTIVE MODELING OF MALARIA ELIMINATION IN GREATER MEKONG SUBREGION

Anchalee Jatapai, Ricardo Aguas, Richard James Maude

Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand

Evidence from mathematical, economic and statistical modelling is becoming increasingly valued for guiding health policy. To ensure modelling is relevant, appropriate and impactful, a strong working partnership between modellers and policymakers is essential. MORU is conducting project "Enhanced modelling for National Malaria Control Programme (NMCP) Decision-making in the Greater Mekong Subregion (GMS) to Accelerate Malaria Elimination (ENDGAME)" in close collaboration with NMCPs and others collaborators in GMS. We are providing technical support with modelling and statistical analysis to help NMCPs optimally target finite resources and maximize their impact and give countries the best chance to achieve malaria elimination goals. We have a major focus on engaging closely with NMCPs who are principal beneficiaries of modelling results to increase understanding of modelling and statistical analysis and ensure relevance, interpretability and usability of outputs. From 2018-2020, we conducted a range of stakeholder engagement activities with NMCPs, broader Ministries of Health, and country/regional partner organizations in GMS to identify gaps in technical assistance. We created a platform for bidirectional sharing of their expertise and experiences and project team using a proactive participatory approach to identify the priority questions and plan timelines of activities. Selection questions process included introductory training in modelling; brainstorming of potential questions; identification of decision points and timelines; questions ranking; assessment of feasibility at different spatial and temporal scales; and selection of appropriate model frameworks; then finalized by the NMCP leadership. To date, the workshops have provided basic training in modelling to over 170 country staff with > 70% of having gained a clear understanding of modelling methods. Stakeholder engagement for ENDGAME is a means of promoting sustainable change for malaria decision-making using predictive modelling through improving use and interpretation of models and by being responsive to the needs and preferences of each country.

753

HEALTH WORKERS' MALARIA CASE MANAGEMENT PRACTICES IN SOUTH CENTRAL UGANDA, 2017-2019

Patricia Mukose[1], Ruth Kigozi[1], Daniella Busharizi[1], Emily Goodwin[1], Gloria Sebikaari[2], Mame Niang[2], Stephen Begumisa[1], Denis Rubahika[3], Thomson Ngabirano[1], James Tibenderana[4], Kassahun Belay[2], Sam Gudoi[1]

[1]*The US President's Malaria Initiative MAPD project, Uganda, Kampala, Uganda,* [2]*US President's Malaria Initiative, US Agency for International Development, Kampala, Uganda,* [3]*Uganda National Malaria Control Division, Kampala, Uganda,* [4]*Malaria Consortium, London, United Kingdom*

The US President's Malaria Initiative's Malaria Action Program for Districts (MAPD) project supports malaria control in nine districts in south-central Uganda. Interventions include healthcare worker malaria case management mentorship and quarterly performance reviews. This study sought to compare health worker practices in project and non-project districts. District Health Information System data from health facilities in the intervention (440) and control areas (303) were analyzed. The study period, January 2017 to December 2019, was divided into a pre-intervention period (pre-int), intervention period with no malaria upsurge (post no upsurge), and an intervention period with malaria upsurge (post upsurge). Three outcomes were assessed: proportion of suspected malaria cases that were tested at outpatient departments, suspected cases with a negative test result treated with antimalarial drugs, and malaria related deaths in inpatient departments. In the intervention area, 82% (895,072/1,090,776) of suspected malaria cases in pre-int were tested, increasing to 92% (1,456,929/1,570,818) in post no upsurge, and 68% (823,322/1,204,987) in post upsurge. In the comparator area this was 65% (756,441 /1,146,156) in both pre-int and post no upsurge, but only 37% (631,731/1,693,818) in post upsurge. The proportion of negative cases treated in the intervention area improved from 34% (156,489/449,119) (pre-int) to 21% (161,445/754,076) (post no upsurge) to 4% (207,02/452,718) (post upsurge), whereas in the comparator area this reduced from 48% (188719 / 385165) to 17% (92618/553831), but rose up to 32% (131428/409374) in times of upsurge. The proportion of malaria deaths reduced from 18.4% (305/1649) (pre-int) to 3.1% (167/5348) (post no upsurge) and to 1.8% (54/2847) (post upsurge) in the intervention area, but increased from 1.7% (93/5461) (post no upsurge) to 3.2% (78/2424) (post upsurge) in the control area. This study suggests that health workers can sustain appropriate case management practices, even in periods of increased workload. These capacity-building efforts could further improve malaria control if scaled up.

754

IMPROVING MALARIA DATA QUALITY FOR PROGRAMMATIC DECISION-MAKING: FINDINGS FROM DATA QUALITY AUDITS IN MAINLAND TANZANIA, 2019

Khalifa Kassim Munisi[1], Pai Chambongo[1], Godfrey Philemon[1], Anna Mahendeka[1], Frank Chacky[1], Abdallah Lusasi[1], Ally Mohamed[1], Ssanyu Nyinondi[2], Shabbir Lalji[3], Humphrey Mkali[2], Joseph Joachim Joseph[2], Chonge Kitojo[4], Naomi Serbantez[4], Erik Reaves[5], Samwel Lazaro[1]

[1]*National Malaria Control Program, Tanzania, Dodoma, United Republic of Tanzania,* [2]*RTI International, Dar es salaam, United Republic of Tanzania,* [3]*RTI International, Dodoma, United Republic of Tanzania,* [4]*US President's Malaria Initiative, United States Agency for International Development, Dar es salaam, United Republic of Tanzania,* [5]*US President's Malaria Initiative, US Centers for Disease Control and Prevention, Dar es salaam, United Republic of Tanzania*

Tanzania has seen a 50% reduction in malaria prevalence in the last 10 years. In 2019, a total of 6,649,333 confirmed malaria cases were reported by 7,950 health facilities nationwide. While the overall reporting rate (99.6%) and completeness (96%) were high, we conducted a malaria data quality audit (DQA) for the 2019 period to verify the routine malaria data reported by health facilities in Mainland Tanzania. Using the outpatient department (OPD) DQA consistency checklist within the Malaria Service and Data Quality Improvement (MSDQI) tool, the number of patients attending OPD, malaria tests performed, and confirmed and clinical malaria cases were compared between the data reported by health facilities in the District Health Information Software (DHIS 2) and recorded in the health facility OPD register. For every matching value, a point was assigned. Health facility scores were aggregated by region; scores >75% were considered acceptable, 50–75% as par, and <50% as unacceptable. In 2019, 26% (2,098) of health facilities had undergone an OPD data consistency check with the MSDQI tool in Mainland Tanzania. Of those, 851 (41%) had an acceptable score >75%, 832 (40%) had a par score between 50–75%, and 415 (19%) had an unacceptable score <50%. In Njombe Region, only one health facility out of 311 for the entire region had undergone an OPD MSDQI DQA in 2019, and its overall performance was <50%. In Mtwara region, among the 88/260 (39%) health facilities that had undergone an OPD MSDQI supportive supervision visit, 42 (48%) had an acceptable DQA score >75%, and only 4 facilities had a score <50%. Disaggregation and review of malaria-related data from DHIS 2 can identify how facilities are performing with consistency of data reported in DHIS 2 and in the OPD registers. Data from the MSDQI tool can be used to guide supportive supervision efforts to address data quality gaps at health facilities.

755

POOR ADHERENCE TO MALARIA TREATMENT GUIDELINES WITH ARTEMETHER-LUMEFANTRINE IN KINSHASA, DEMOCRATIC REPUBLIC OF CONGO

Kristin E. Banek[1], Mvuama M. Nono[2], Melchior Kashamuka Mwandagalirwa[2], Georges Emo Mahilu[2], Joseph A. Bala[2], Georges Kihuma[2], Joseph Atibu[2], Marthe Nkalani[2], Tommy Nseka Mambulu[2], Jonathan J. Juliano[1], Antoinette Kitoto Tshefu[2], Jonathan B. Parr[1], Steve R. Meshnick[1]

[1]*University of North Carolina-Chapel Hill, Chapel Hill, NC, United States,* [2]*Ecole de Santé Publique, Faculté de Médecine, University of Kinshasa, Kinshasa, Democratic Republic of the Congo*

A cornerstone of malaria control in the Democratic Republic of Congo (DRC) is confirmatory diagnosis and effective case management with artemisinin-based combination therapy (ACT). However, despite the adoption of these effective case management strategies, implementation challenges remain. As part of an ongoing longitudinal malaria study of 1,600 subjects in Kinshasa Province, all positive rapid diagnostic test (RDT) participants were tracked and followed during two biannual active surveillance surveys. Data were abstracted from the health facilities to determine if a participant received artemether-lumefantrine (AL). One week later, participants were visited at home to measure treatment initiation, dosing procedures, and treatment completion rates. Initial analysis included 957 participant interviews from two health areas. Half of the participants had a positive malaria test, with only 13% of participants reported having fever the week before the survey. At the one-week follow-up visit, 95% of test-positive participants were interviewed. Only 57% of all RDT-positive participants collected treatment from the local health facility, but access varied by health area (53% vs. 61%; p=0.04). The most common reasons given for not collecting treatment were: too busy/unavailable (46%), no reason/indifference (19%), forgot (7%), health facility or medication reasons (15%), and other (12%). All participants collecting treatment received AL, and 97% initiated treatment. Initiation was significantly different by health area (100% vs. 93%; p=0.002). Treatment completion was 81% among those initiating AL, with no differences between areas. Overall, only 45% of RDT-positive participants referred for treatment received and completed treatment. While treatment completion was exceptional among participants that started their treatment, significant barriers remain in accessing and initiating treatment. These findings illustrate the chasm between malaria treatment guidelines and practice in the DRC and emphasize the need to improve the malaria treatment cascade and understand factors associated with these barriers.

757

UNDERSTANDING MALARIA PREVENTIVE BEHAVIORS AMONG THE CROSS-BORDER POPULATION ALONG THE THAI-MYANMAR BORDER IN TAK PROVINCE, THAILAND

Siriporn Yongchaitrakul[1], Daniel M. Parker[2], Pratap Singhasivanon[1], Liwang Cui[3], Jetsumon Sattabongkot[1], Suparat Phuanukoonnon[1]

[1]*Mahidol University, Bangkok, Thailand,* [2]*University of California, Irvine, Irvine, CA, United States,* [3]*University of South Florida, Tampa, FL, United States*

Malaria transmission in the GMS exhibits extreme spatiotemporal heterogeneity and malaria clusters in hotspots. These remaining reservoirs tend to be located within specific ecologies, where risk of ongoing transmission is contingent on human behavior patterns. Demographic groups at high risk for malaria include those who travel to the field or forest and/or stay outside at night and without bed nets. This research aimed to explore preventive behavior patterns to malaria infection among those who crossed the Thailand-Myanmar border and/or stayed overnight in Myanmar. A mixed methods approach was used, including a KAP questionnaire, diaries recording daily travel and mosquito bite prevention and in-depth interview from March to October 2019. The study was carried out in four villages in the Tha Song Yang District of Tak Province, on the Thai side of Thai-Myanmar border, adjacent to the Moi River. This site has been part of studies led by the International Centers of Excellence for Malaria Research (ICEMR) project since 2011. The results of KAP questionnaire survey covered 386 participants and showed common misunderstandings of the causes of malaria infection (e.g., drinking water from mosquito breeding place) and malaria vector biting times; while bed net and repellent were well regarded as effective preventive tools. The diaries showed that going to forest was the main reason for cross-border travel to Myanmar and males crossed the border two-times more frequently than females. Approximately 60% of participants who crossed the border had practiced at least one of these preventive methods (e.g., wearing long sleeve cloths, expelling mosquito/insect with wood smoke or cigarette smoking, sleeping under bed net or blanket, and applying repellent) depending on their habits and mosquito abundance. Conversely 20% irregularly practiced and a further 20% did not do any prevention. In conclusion, misunderstandings about malaria persist despite decades of malaria health education program.

758

ASSESSING THE RELIABILITY OF SURVEILLANCE DATA COLLECTED BY DISTRICT MALARIA SURVEILLANCE OFFICERS IN UNGUJA, ZANZIBAR - 2019

Wahida S. Hassan[1], Abdullah Ali[1], Mohamed Ali[1], Mwatima Suleiman[1], Makame Makame[1], Amina Faki[1], Raya Ibrahim[1], Salum Massoud[1], Naomi Serbantez[2], Chonge Kitojo[2], Humphrey Mkali[3], Shabbir Lalji[3], Nyinondi Ssanyu[3], Erik Reaves[4], Abdul-wahid Al-mafazy[5]

[1]*Zanzibar Malaria Elimiantion Programme, Zanzibar, United Republic of Tanzania,* [2]*US President's Malaria Initiative, United States Agency for International Development, Dar es Salaam, Tanzania, Dar-es-salaam, United Republic of Tanzania,* [3]*RTI International, Dar-es-salaam, United Republic of Tanzania,* [4]*US President's Malaria Initiative, Centers for Disease Control and Prevention, Dar es Salaam, Tanzania, Dar-es-salaam, United Republic of Tanzania,* [5]*RTI International, Zanzibar, United Republic of Tanzania*

Zanzibar introduced malaria case-based surveillance in 2012. District Malaria Surveillance Officers (DMSOs) investigate malaria cases notified by health facilities. Using a web-based surveillance system, DMSOs collect index case details at the diagnosing heath facility and household, perform malaria rapid diagnostics tests (mRDT) for members of index case households, treat those with a positive mRDT result with antimalarial drugs, and complete case classification based on WHO guidelines. These data are transmitted to a central database for analysis and use. However, the reliability of this information is unknown. We aimed to assess the consistency of surveillance data elements and accuracy of case classification. Between August and September 2019, Zanzibar Malaria Elimination Program staff extracted data from the central database on 60 randomly selected index cases previously investigated by 16 DMSOs in all 7 districts of Unguja. The study team visited the health facilities where index cases sought treatment and their households to collect data using a structured checklist to compare data consistency and accuracy on investigations of index cases and respective household members, mRDT results, and case classification reported by DMSOs. Consistency was evaluated by comparing results reported by DMSOs to results recorded by the study team. Accuracy was defined as the percent of correctly captured data and classification of cases by DMSOs. The study team confirmed DMSOs investigated all 60 (100%) index cases at both health facility and household levels. Overall consistency between data reported by DMSOs and the study team was 90% (range 54-98%). The accuracy of data capture and case classification was 95% (range 71-99%), with 33 (58%) cases classified as imported and 24 (42%) as locally acquired. Reported completion of mRDT testing for household members by DMSOs was 43% (range 17-64%) compared to those eligible 47% (range 17-68%) (P=0.570). Our findings suggest data reported by DMSOs are consistent and accurate, and therefore reliable for decision-making. Barriers to low testing rates in households should be investigated.

759

INFANT SEX MODIFIES ASSOCIATIONS BETWEEN PLACENTAL MALARIA AND RISK OF MALARIA IN INFANCY

Abel Kakuru[1], Michelle Roh[2], Richard Kajubi[1], Teddy Ochieng[1], John Ategeka[1], Harriet Ochokoru[1], Miriam Nakalembe[3], Tamara D. Clark[2], Theodore Ruel[2], Sarah G. Staedke[4], Daniel Chandramohan[4], Diane V. Havlir[2], Moses R. Kamya[3], Grant Dorsey[2], Prasanna Jagannathan[5]

[1]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [2]*University of California San Francisco, San Francisco, CA, United States,* [3]*Makerere University College of Health Sciences, Kampala, Uganda,* [4]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [5]*Stanford University, San Francisco, CA, United States*

Effective intermittent preventive treatment of malaria in pregnancy (IPTp) has been associated with a lower malaria incidence in infants during the first year of life. However, it is unclear whether this association is mediated through the prevention of placental malaria (PM) during pregnancy. We assessed for the association between PM and the incidence of malaria during the first year of life, and for the mediation effect of PM in the association between IPTp and the incidence of malaria infants in a birth cohort of 656 infants born to HIV-uninfected mothers randomized to IPTp with dihydroartemisinin-piperaquine (DP) versus sulfadoxine-pyrimethamine (SP). PM was defined as the detection of malaria parasites or pigment by histology (n=292). PM was further categorized by the proportion of high-power fields (HPFs) with malaria pigment deposited in fibrin: as no pigment (n=351); mild-moderate (>0-20% HPFs with pigment, n=218); and severe (>20% HPFs with pigment, n=70). There was no association between PM detected by histology and incidence of malaria in infants (adjusted incidence rate ratio [aIRR] 1.00, 95% confidence interval [CI] 0.83-1.21, p=0.99). However, compared to no pigment deposition in fibrin, severe pigment deposition was associated with higher incidence malaria during infancy (aIRR 1.22, 95% CI 0.88-1.69, p=0.24) which was statistically significant only in male infants (aIRR 1.83, 95% CI 1.22-2.74, p<0.003), but not female infants (aIRR 0.77, 95% CI 0.49-1.21, p=0.26). Among male infants, 53% of the reported protective effect of IPTp-DP on the incidence of malaria in infants was attributed to IPTp-DP's greater effect on reducing the severity of PM compared to SP. Our findings suggest that the severity of pigment deposition is associated with malaria in infancy but only in male infants implying that prevention of severe PM may be protective among male infants. More research is needed to understand the mechanism of this sex difference and to evaluate the association between PM and malaria in infants residing in moderate malaria transmission settings.

760

QUALITY OF CARE FOR CHILDREN WITH MALARIA AT PRIVATE HEALTH FACILITIES IN THE MIDWESTERN REGION OF UGANDA: A CROSS SECTIONAL STUDY

Patrick Bukoma[1], Humphrey Wanzira[2], Daniel Tumwine[2], Allan Musiime[2], Juliet Biculi[2], Tom Ediamu[2], Rebecca Nantanda[2], Emily Goodwin[1], Jane Achan[2], Ronald Mulebeke[2], James Tibenderana[3], Gloria Sebikari[4], Mame Niang[5], Kassahun Belay[4]

[1]*Malaria Consortium, Kampala, Uganda,* [2]*Uganda Paediatric Association, Kampala, Uganda,* [3]*Malaria Consortium, London, United Kingdom,* [4]*US President's Malaria Initiative, US Agency for International Development, Kampala, Uganda,* [5]*US President's Malaria Initiative, Malaria Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA, Kampala, Uganda*

The 2018 Uganda Malaria Indicator Survey showed that 59% of the population seek advice or treatment from the private sector. Despite this, national efforts in malaria-related capacity building have centered on public facilities, and the quality of care within Uganda's private health facilities remains largely undocumented. This study assessed the quality of malaria-related care services in private facilities operating in districts supported by the US President Malaria Initiative's Malaria Action Program for District project in Uganda. In October 2018, a cross-sectional study using qualitative and quantitative interviews was conducted in 134 private health clinics and one hospital, purposively sampled from nine districts in the Mid-Western region of Uganda. Of the studied facilities, 61.5% had access to and used treatment protocols while 48.9% had received malaria management training. 98.5% had malaria laboratory services, but only 57.8% had qualified laboratory personnel. 77.8% had experienced stock-outs of anti-malarials in the previous 3 months. 14.1% of health workers responded correctly to questions on clinical and preventive treatment of malaria. 33.3% responded correctly to fever management questions, 40.0% correctly identified first-line treatment for uncomplicated malaria, and 85.2% for complicated malaria. Only 28% of the facilities submitted monthly data to the national health information management system. Qualitative interviews identified that lack of: access to national quality assurance tools, health worker training and supportive supervision, and essential anti-malaria commodities affected performance. Knowledge and practices of private facility health workers within this region are poor. This can increase malaria-related morbidity and mortality risks. Low reporting

rates makes accurately assessing Uganda's malaria situation difficult and poses a challenge for the effective planning and implementation of a national malaria program. Interventions aimed to improve malaria quality of care in private facilities would build individual as well as system capacities.

761

IMPLEMENTING QUANTITATIVE POINT-OF-CARE G6PD TESTING IN BANGLADESH: INSIGHTS FROM A TRAINING WORKSHOP AND QUALITATIVE RESEARCH STUDY

Sandra Incardona[1], Rosalind E. Howes[1], Nora Engel[2], Cristian Ghergu[2], Mohammad S. Alam[3], Mohammad G. Kibria[3], Abdul Matin[3], Kamala Thriemer[4], Benedikt Ley[4], Sabine Dittrich[1], Xavier C. Ding[1]

[1]*FIND, Geneva, Switzerland,* [2]*University of Maastricht, Maastricht, Netherlands,* [3]*iccdr,b, Dhaka, Bangladesh,* [4]*Menzies School of Health Research, Darwin, Australia*

8-aminoquinolines effectively remove dormant *Plasmodium vivax* liver stages from the human host and are essential for the control and elimination of vivax malaria. Well tolerated in most patients, they can cause severe hemolysis in G6PD deficient patients, and the WHO therefore recommends routine testing to guide treatment. All point of care (PoC) G6PD diagnostics currently in use on a routine basis provide a qualitative output, however the introduction of novel short course treatment options will require a quantitative diagnosis. A handheld quantitative assay (STANDARD™ G6PD) is now available, with performance that may render the device suitable for integration into routine practice. We assessed perceptions on the ease of use and feasibility to include the device in routine care in Bangladesh. A total of 29 health workers (HWs) from primary health facilities and six national reference laboratory technicians were trained on G6PD testing using the STANDARD™ G6PD test and included in subsequent focus-group discussions (FGDs). Semi-structured interviews were also conducted with 20 of the trained HWs in their places of work and eight public health experts, to understand perspectives on the test's ease of use and future implementation. The analysis of the interviews and FGDs revealed that the device's portability and its capacity to provide both hemoglobin and G6PD results were appreciated, while complexity and handling, in comparison with malaria RDTs, were noted as downsides. Interviewees were also concerned about supply chains and shelf life, and discussed operational aspects such as centralized versus PoC testing, and the challenge of justifying more investment and workload in the context of decreasing malaria burden. The study provides useful insights about barriers that would have to be overcome and factors that would facilitate implementation, both from a practical point of view at HWs level, and a more strategical and logistical perspective at programme level. The lessons learned could help designing an efficient implementation plan in Bangladesh but also be useful for other countries with similar settings and challenges.

762

SUSTAINING IPTP 3 UPTAKE IN MALAWI THROUGH A HEALTH SYSTEMS APPROACH

John Munthali[1], Evans Kaunda[2], Kate Wright[3], Tom Hall[4]

[1]*Management Sciences for Health, Lilongwe, Malawi,* [2]*Ministry of Health, Malawi, Lilongwe, Malawi,* [3]*Management Sciences for Health, Medford, MA, United States,* [4]*Management Sciences for Health, Arlington, VA, United States*

In 2013, based on the World Health Organization's updated policy on intermittent preventive treatment in pregnancy (IPTp), Malawi adopted the new recommendation of three or more doses of sulfadoxine-pyrimethamine during pregnancy. Support from the President's Malaria Initiative (PMI), working through USAID funded health projects - SSDI (2012-2016) and ONSE Health Activity (2016-2020) - enabled collaboration between the National Malaria Control Program (NMCP) and the Reproductive Health Directorate (RHD) of the Ministry of Health to build capacity to implement the updated IPTp policy. Significant gains in IPTp3 uptake have been observed following the policy change, starting with the 2014 Malawi Malaria Indicator Survey (MIS) IPTp3 was at 13%, which increased to 30% in the 2015 Malawi Demographic Health Survey, and increased to 43% in the 2017 MIS. Sustaining such achievements requires a concerted, systems approach. ONSE, with PMI funding, is collaborating with the NMCP and RHD to sustain and increase IPTp3 uptake in 16 districts. At the community level, ONSE is awarding small grants to NGOs to improve uptake of malaria services. Community volunteers and local community-based organizations are engaged in order to identify and solve local problems, as well as encouraging pregnant women to attend antenatal care and receive malaria prevention services. At the facility level, ONSE is supporting frequent and targeted supportive supervisions, coaching and mentoring to ensure compliance to the new IPTp guidelines, and at national level, supporting the Malaria in Pregnancy Technical Working Group to meet quarterly, which includes NMCP, RHD, implementing partners and academia as members. According to DHIS2 data, IPTp 3 uptake has improved to 46% in 2018 and 50% in 2019. Malawi's experience shows that collaboration between various stakeholders and strengthening various levels of health services can lead to improved knowledge of the new policy, resulting in increased uptake of services and protection of pregnant women from malaria.

763

EVALUATING THE IMPACT OF SEASONAL MALARIA CHEMOPREVENTION (SMC) ON CHILDREN UNDER-FIVES' MALARIA TEST POSITIVITY RATES (TPR): A COMPARATIVE STUDY OF EIGHT GLOBAL FUND SUPPORTED LOCAL GOVERNMENT AREAS (LGA) ACROSS KATSINA & YOBE STATES, NIGERIA, 2017 - 2019

Akinola Shonde[1], Olabisi Kalejaye[2], Temitope Ipinmoye[1], Yakubu Cherima[3], Chinedu Chukwu[2], Temitope Adeyoju[1], Chidinma Okolo[1], Perpetua Uhomoibhi[4], Diwe Ekweremadu[1], Sonachi Ezeiru[1], Olugbenga Mokuolu[4]

[1]*Catholic Relief Services Nigeria, Abuja, Nigeria,* [2]*Management Sciences for Health, Abuja, Nigeria,* [3]*Malaria Consortium, Abuja, Nigeria,* [4]*National Malaria Elimination Program, Abuja, Nigeria*

Seasonal malaria chemoprevention involves cyclical mass administration of Sulphadoxine - Pyrimethamine & Amodiaquine during rainy season in sahel sub-region, for malaria prevention amongst children 3 - 59 months old. In 2019; with support from the Global Fund, SMC was newly implemented in 4 LGAs each in Katsina (Daura, Jibia, Kaita, Zango) & Yobe (Bade, Karasuwa, Nguru, Machina) states to target 714,992 children in Nigeria. This study measures the impact of SMC on malaria burden during high transmission season (July - October) in 8 LGAs across the 2 states. The TPR amongst children <5 & ≥5 years were analyzed from 2017 (no SMC in the LGAs) - 2019 (during SMC implementation); & across LGAs (SMC-implemented & non-implemented LGAs). Evaluation was done using secondary data set on district health information system, DHIS-2 from Aug - Nov. in 2017, 2018 & 2019. A desk review & analysis of 2017 & 2018 national health management information system, NHMIS data was carried out to establish baseline TPR for children <5 in focal LGAs; this was compared with TPR in same cohort during 2019 SMC implementation period in both States. While a rise in TPR was observed from 2017 - 2018, there was a decline in TPR across the 8 LGAs in both States from 2018 - 2019 as shown - Katsina; Daura (10%), Jibia (10%), Kaita (2%) & Zango (25%). - Yobe; Bade (15%), Karasuwa (9%), Machina (4%) & Nguru (10%). Cumulatively, the 4 LGAs each in Katsina and Yobe recorded an average of 12% & 10% decline in TPR, respectively between 2018 & 2019 following SMC intervention. SMC could have contributed to this drop as suggested by findings following analysis of same data in 4 other non-contiguous but eligible LGAs where SMC was not implemented. There was a cumulative decline in TPR by 5% in Katsina & an increase in TPR by 7% in Yobe during the same study period. No significant reduction in TPR was observed following analysis of same data for ≥5 years age group during the same period. This study provides evidence of early gains from SMC &

can therefore be anticipated that more SMC rounds will further reduce parasite's burden. For a more comprehensive evaluation of SMC impacts, there will be a need for a population-based assessment.

764

FINDINGS OF THE INDEPENDENT SURVEY OF THE SEASONAL MALARIA CHEMOPREVENTION CAMPAIGN IN MOPTI AND SEGOU REGION IN MALI IN 2019

Beh Kamate[1], Mohamadine A. Maiga[1], Renion Saye[1], Samba Coumare[1], Oumar Yattara[1], NénéBoua Konaté[1], Boubacar Guindo[1], Curt von Boguslawski[2], Jules Mihigo[3], Aliou Diallo[4], Kathryn Malhotra[5], Charlotte Eddis[5]

[1]*PMI Impact Malaria, Bamako, Mali,* [2]*Population Services International, Bamako, Mali,* [3]*U.S. President's Malaria Initiative, Bamako, Mali,* [4]*U.S. President's Malaria Initiative, Bamako, Mali,* [5]*PMI Impact Malaria Project, PSI, Washington, DC, United States*

In 2019, the National Malaria Control Program (NMCP) in Mali, in collaboration with its partners, conducted an independent survey at community level to evaluate the implementation of the Seasonal Malaria Chemoprevention (SMC) campaign. The US President's Malaria Initiative Impact Malaria project supported the NMCP to organize this survey in Mopti and Segou regions (others supported the same survey in other regions). The survey was conducted after the 1st and 4th campaign cycles (in August and November). Two districts per region were selected based on high and low reported coverage rates. Within each district, two health areas were selected: the main urban area and one randomly selected rural area. In total, 32 villages and 1,280 family compounds were selected. Caregivers of children who had received SMC were interviewed and a total of 1,413 people were surveyed in the two regions. Data were collected using electronic tablets and analyzed in SPSS. The average administrative coverage rate was 103% over the four cycles in Mopti and Segou regions, as reported by NMCP. However, the results of the survey done after the 4th cycle indicated lower coverage rates: according to the caregivers' declaration, 94% of children were treated with SP-AQ during all four cycles, but only 73% of caregivers could show proof that their children had received SP-AQ four times (i.e. through SMC cards and drug blister-packs). Anecdotal evidence indicated that the difference could be explained by non-availability and/or loss of SMC card or finished blister-packs. Caregivers declared that 92% of children received the second and third doses at home. This was true for both the 3-11- and 12-59-month age groups (n=311 and n=1102 respectively) and for both regions. 41% of caregivers reported receiving SMC messages from town criers and 25% from community radio. The survey made it possible to assess the progress of the campaign while it was underway and adjust for any future cycles as needed. Coverage was high, although there was a disparity between parent and administrative reports, which could be explored more in future years' surveys. Reported adherence for second and third doses was high.

765

ASSESSMENT OF PHARMACOVIGILANCE REPORTING IN SEASONAL MALARIA CHEMOPREVENTION IN THE SAHEL REGION OF NIGERIA

Jimmy Aiden[1], Kenneth Maduka[2], Mujahid Idris[2], Chrysanthus Dabes[3], Obinna Emeruwa[4], Abba Sagagi[1], Taiwo Ibinaiye[5], Bello Magaji[5], Babatunde Fagbemi[5], Kunle Rotimi[5], Abimbola Phillips[5], Olatunde Adesoro[5], Olusola Oresanya[5]

[1]*Malaria Consortium, Dutse, Nigeria,* [2]*Malaria Consortium, Zamfara, Nigeria,* [3]*Malaria Consortium, Katsina, Nigeria,* [4]*Malaria Consortium, Sokoto, Nigeria,* [5]*Malaria Consortium, Abuja, Nigeria*

Pharmacovigilance (PV) in mass drug administration strategies such as seasonal malaria chemoprevention (SMC) involves identification and reporting of suspected adverse drugs reactions (ADR). Although PV is a core component of SMC, this has not been well evaluated in Nigeria, where over four million eligible children benefited from SMC in 2019. SMC implementation includes a referral and reporting system for SMC-related ADR from communities, involving health facilities (HFs) and National Agency for Food and Drug Administration and Control (NAFDAC) state offices. Malaria Consortium in collaboration with Government of Nigeria trained health facility workers (HFWs) on PV before the start of SMC. A commodity management audit (CMA) in December 2019 collected data from 1,233 randomly selected HFs in five SMC intervention states - Jigawa, Katsina, Sokoto, Yobe, and Zamfara. Secondary analysis of CMA data from 1,127 HFs assessed availability of PV reporting tools, HFWs trained on the tools and compliance with the standard ADR reporting protocol. PV forms were available in 84% of the health facilities sampled and 91.6% had at least one HFW trained on PV for the SMC campaign. This varied across the states from 95.6% to 67.7% for reporting tools and 97.3% to 79.2% for HFWs trained on the tools. Nine percent of health facilities identified and documented at least one suspected ADR during SMC, the commonest being vomiting. Of these HFs, only 19.8% reportedly sent their PV forms to NAFDAC state office. ADR reporting varied by state, from 4.7% to 100%, but was not associated with reporting form availability or training, suggesting the reason could be behavior based. This study indicates the institutionalization of PV into SMC implementation, with availability of trained HFWs and reporting forms at service delivery points. However, ADR reporting by HFWs is still a challenge. Thus, close attention should be given to this during routine supervision to strengthen compliance to the standard reporting processes and to encourage active PV screening, such that every suspected ADR is identified, documented and submitted to NAFDAC for further action and feedback.

766

BRINGING LONG-LASTING INSECTICIDAL NETS (LLIN) DISTRIBUTION CLOSER TO COMMUNITIES IN GUINEA THROUGH ADAPTIVE MANAGEMENT

Aissata Fofana[1], Mohamed Sitan Keita[1], Hamidou Barry[1], Mohamed Saran Condé[1], Lamine Bangoura[2], Eugène Kaman Lama[3], Alioune Camara[3], Donal Bisanzio[4], Elizabeth Fitch[5]

[1]*RTI International, Conakry, Guinea,* [2]*President's Malaria Initiative, US Agency for International Development, Conakry, Guinea,* [3]*National Malaria Control Program, Ministry of Health, Conakry, Guinea,* [4]*RTI International, Washington, DC, United States,* [5]*RTI International, RTP, NC, United States*

The use of long-lasting insecticidal nets (LLIN) to prevent malaria has been a vital tool in the global reduction of malaria. To significantly reduce the transmission of malaria, Guinea's National Malaria Control Program (NMCP) included free LLIN distribution campaigns every 3 years in their national malaria control plan. However, in 2018 only 30.7% of the population in Guinea had access to a LLIN. In 2019, the President's Malaria Initiative (PMI) funded StopPalu+ project to support the NMCP distribute LLINs in 19 of the country's 38 districts. An adaptive management approach was used to reach the most at-risk communities. The first phase of distribution was in Labé region and four districts in Boké. At the end of the second day of distribution, routine monitoring data showed that in half of the districts, there was a lower daily household coverage rate (between 11% and 17%) compared to the 2016 LLIN campaign (over 20%). The StopPalu+ team contacted the local authorities (mayors, neighborhood leaders, and religious leaders) to understand why people were not coming to the distribution site. Qualitative feedback from discussions with local authorities indicated that the distribution points were too far for people to reach after their agriculture work at 5PM prior the distribution point closing at 7PM. Additionally, many households had lost their vouchers and were afraid to come to the sites without it. By triangulating monitoring data and using qualitative findings, the team decided to split the distribution points and move half the staff closer to the communities that had the lowest attendance. Radio spots were also produced to encourage people to provide their names to get a copy of their vouchers and a LLIN. With these changes, the percentage of households served per day increased to the expected 20%. The revised strategy was implemented in the subsequent regions and achieved the daily expected coverage rate with an overall LLIN coverage of 95% of

the targeted population. This case demonstrates the importance of adaptive management and community engagement to provide targeted populations with products and services to ensure adequate access.

767

PROTOCOL ADHERENCE IN SEASONAL MALARIA CHEMOPREVENTION AND FEVER OCCURRENCE AMONG UNDER-FIVE CHILDREN IN NORTHERN NIGERIA

Abimbola Phillips[1], Olusola Oresanya[1], Olatunde Adesoro[1], Taiwo Ibinaiye[1], Rilwanu Anka Mohammed[1], Roni Babangida Lawal[1], Sherifat Ibrahim[1], Azuka Iwegbu[1], Jamilu Ibrahim Nikau[2], Maxwell Kolawole[1], Robinson Daniel Wammanda[3]

[1]*Malaria Consortium, Abuja, Nigeria,* [2]*National Malaria Elimination Programme, Abuja, Nigeria,* [3]*Ahmadu Bello University, Zaria, Nigeria*

Fever is the commonest symptom of malaria among under-5 children living in malaria endemic regions, and often used as a proxy for assessing malaria morbidity and impact of malaria control interventions. Seasonal malaria chemoprevention (SMC), an effective malaria control intervention treating children aged 3-59 months in the Sahel, is delivered by community health workers (CHWs) guided by a standard protocol. However, there have been concerns about the quality of SMC service delivery by CHWs, which includes delivery of three key messages on administration of all SMC doses, redosing if child vomits and child sleeping under mosquito net. About 4 million children in five northern Nigeria states (Jigawa, Sokoto, Katsina, Yobe and Zamfara) received SMC between July-October 2019. Using data from the December 2019 household survey covering 5,215 eligible children, we assessed the CHWs' adherence to the protocol, examined the association with community-based fever reports, and investigated variations related to additional use of other malaria control measures. The study outcome was reported fever within the last month prior to the survey. The level of protocol adherence by CHWs as reported by caregivers was categorised as 1) High - directly observed treatment (DOT) and three messages given; 2) Medium - only DOT; and 3) Low – no DOT. Odd ratios adjusted for various factors including use of additional malaria control measures were estimated. About 43% of SMC-treated children had fever in the last month prior to survey. Among children surveyed, CHW protocol adherence was high for 12.5%, medium for 57.6%, and low for 29.9%. The odds of fever occurrence were significantly lower with high (AOR = 0.61; p = 0.04) and medium adherence (AOR = 0.75; p = 0.03) compared to low protocol adherence (test for trend: p=0.012). In addition, we found a cumulative effect in reducing fever occurrence when the child slept under mosquito net. SMC impact on fever is therefore likely to be critically linked to quality of intervention delivery and this should be emphasized in CHW training and supervision. Additional malaria control measures are needed to improve the effectiveness of SMC.

768

TRENDS OF INTERMITTENT PREVENTIVE THERAPY UPTAKE IN PREGNANT WOMEN ATTENDING ANTENATAL CARE, 2015 TO 2019, MAINLAND TANZANIA

Samwel Lazaro[1], Khalifa Munisi[1], Franky Chacky[1], Anna Mahendeka[1], Ssanyu Nyinondi[2], Shabbir Lalji[2], Humphrey Mkali[2], Joseph J. Joseph[2], Chonge Kitojo[3], Naomi Serbantez[3], Erik Reaves[4], Claud John[5], Ally Mohamed[1]

[1]*National Malaria Control Programme, Dodoma, United Republic of Tanzania,* [2]*RTI International, Dar es Salaam, United Republic of Tanzania,* [3]*US President's Malaria Initiative, United States Agency for International Development, Dar es Salaam, United Republic of Tanzania,* [4]*US President's Malaria Initiative, US Centers for Disease Control and Prevention, Dar es Salaam, United Republic of Tanzania,* [5]*Ministry of Health Community Development Gender Elderly and Children, Dodoma, United Republic of Tanzania*

Tanzania introduced Intermittent Preventive Therapy in pregnant women (IPTp) in 2002. Currently it covers 6,935 antenatal clinics (ANC) across the country and targets all pregnant women attending ANCs, using sulfadoxine pyrimethamine (SP) as drug of choice. The World Health Organization recommends that pregnant woman receive at least three doses of SP during gestation period, from second trimester with an interval of one month apart, in order to minimize malaria complications in mothers and neonates. The National Malaria Control Program assessed trends of IPTp uptake to determine progress in meeting the national target of 80% coverage for IPTp2 and IPTp3. IPTp uptake data reported by health facilities were downloaded from Ministry's District Health Information Software 2 (DHIS2) system for all 26 regions for the period of 2015 to 2019. Data were cleaned and analyzed using Microsoft Excel. An average of 89,296 pregnant women made at least one ANC visit annually. Overall, there was a 30-percentage point increase in IPTp2 uptake from 57% in 2015 to 87% by 2019. In 2019, Kilimanjaro and Tabora regions had the lowest (75%) and highest (100%) IPTp2 uptake respectively. IPTp3 was introduced in December 2015 with an uptake of 2%. By 2019, among 2,321,703 pregnant women who attended at least one ANC visit, overall uptake was 71%, with Tabora region having the lowest (58%) and Kilimanjaro region having the highest (94%) uptake. DHIS2 data look at coverage only among women attending at least 1 ANC, thus tend to have higher coverage than surveys; in 2017, the malaria indicator survey found 25.5% IPTp3 coverage, while DHIS2 found 32.6%. This assessment shows that there has been steady progress in IPTp2 uptake in Mainland Tanzania, as it maintained well above the national target in the last two years. Likewise, IPTp3 shows substantial improvement, but coverage in most regions remains below the national target. Further investigation is required, especially in health facilities that consistently report low IPTp coverage. This may be due to periodic stock outs of SP, poor retention in ANC, or readiness of health facilities and staff to provide the relevant services.

769

CALL FOR IMPROVING ACCESS TO MALARIA DIAGNOSIS AND TREATMENT IN UNDERSERVED RURAL AND REMOTE ENDEMIC AREAS OF TANZANIA

Issa Ally Garimo[1], Abdallah Lusasi[1], Ally Mohamed[1], Samwel Lazaro[1], Chonge Kitojo[2], Goodluck Tesha[3], Joseph Mugasa[4], Godfery Smart[5], Emmanuel Kipande[6], Julian Mgengi[7], Kibua Kakolwa[8], Fabrizio Molteni[9]

[1]*National Malaria Control Program, Dodoma, United Republic of Tanzania,* [2]*US President's Malaria Initiative/Centers for Disease Control & Prevention, Dar es Salaam, Tanzania, Dar Es Salaam, United Republic of Tanzania,* [3]*USAID Boresha Afya lake and western zone, Tanzania, Mwanza, United Republic of Tanzania,* [4]*USAID Boresha Afya Southern zone Tanzania, Dar Es Salaam, United Republic of Tanzania,* [5]*Regional Health Management Tea, Kigoma, United Republic of Tanzania,* [6]*Regional Health management Team, Katavi, United Republic of Tanzania,* [7]*Regional Health Management Team, Kagera, United Republic of Tanzania,* [8]*Regional Health Management Team, Ruvuma, United Republic of Tanzania,* [9]*Swiss Tropical and Public Health, Dar Es Salaam, United Republic of Tanzania*

Malaria Community Case Management (mCCM) promotes the early recognition, prompt testing and appropriate treatment of malaria. Generally, mCCM has evolved into a more comprehensive strategy that addresses the other two main childhood killers in Africa: pneumonia and diarrhoea through the integrated community case management (iCCM). In Tanzania, policy do not allow diagnosis and treatment services outside the operational healthcare facilities. The National Malaria Control Program (NMCP) is planning to introduce targeted mCCM interventions in phases and to create an enabling environment to expand the scheme towards the two remaining iCCM components. Five regions selected based on two major criteria: high malaria burden and low access to healthcare. Projection for 2019 of the 2012 general census population were estimated for each village. Villages distance from the nearest health facility obtained from the respective council authorities. Distance was assigned to the following categories: within 5km, 5.1 to 10km and above 10kms. Malaria burden, categorized as very low, low, moderate and high, was calculated from three health facility generated indicators: annual incidence rate, malaria positivity rate and positivity rate in pregnant women attending

ante natal clinic. In all five selected region, 270 villages (13%) are located more than 10km from nearby health facility. A total of 847,791 people equals to 10% of all population in five regions are traveling more than 10km to access health facility. More than 90% (797) of all health facilities in these five regions have high and moderate malaria burden In the 5 regions assessed, about 10% of the population living in high and moderate malaria risk areas have low access to prompt malaria diagnosis and treatment services. There is a need to improve accessibility to malaria services by implementing community case management to offer quality and timely malaria services to all people living in underserved rural areas.

770

EFFECTIVENESS OF LONG LASTING INSECTICIDAL NETS AND INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY UPTAKE AMONG PREGNANT WOMEN ATTENDING ANTENATAL CARE IN FIVE REGIONS OF UGANDA

Emily Goodwin[1], Sam Gudoi[1], Ruth Kigozi[1], JohnBaptist Bwanika[1], Thomson Ngabirano[1], Jane Nabakoza[2], Paul Mbaka[3], Joel Kisubi[4], Mame Niang[4], Kassahun Belay[4], Gloria Sebikaari[4], James Tibenderana[5]

[1]*The US President's Malaria Initiative MAPD project, Uganda, Kampala, Uganda,* [2]*Uganda National Malaria Control Program, Kampala, Uganda,* [3]*Ministry of Health, Kampala, Uganda,* [4]*US President's Malaria Initiative, US Agency for International Development, Kampala, Uganda,* [5]*Malaria Consortium, London, United Kingdom*

The US President Malaria Initiative's Malaria Action Program for District project has supported the Ugandan National Malaria Control Division to strengthen the country's Malaria in Pregnancy (MIP) program since 2017. Previous studies have documented successes in uptake of MIP prevention measures, however the programmatic linkage between these interventions and their effect on number of MIP cases is still limited. To investigate the effect of MIP preventative approaches in Uganda relative to trends of MIP cases, this study investigated the relationship between the number of MIP cases and the uptake of prevention interventions among pregnant women attending antenatal care. The study assessed health facility data in the projects five regions between January 2017 and December 2019. Data on MIP cases, uptake of three or more doses of intermittent preventive treatment in pregnancy (IPTp3+), and women receiving long lasting insecticidal nets (LLINs) as a proportion of those attending the first antenatal care visit (ANC 1) was analysed. IPTp3+ increased from 5% in 2017, to 37% in 2018, to 64% in 2019. Similarly, those receiving a LLIN at ANC1 increased from 48% to 66% to 79%. Correlation analysis showed plausible trends between increasing IPTp3+ rates and decreasing MIP cases in four regions (Bunyoro (r=-0·04, p=0·910), Rwenzori (r=-0·25, p=0·425), Kampala (r=-0·13, p=0·693) and Masaka (r=-0·8, p=0·002)). In these same regions, correlation trends between increasing LLIN uptake at ANC 1 and decreasing MIP cases were also seen (Bunyoro (r=-0·25, p=0·419), Rwenzori (r=-0·37, p=0·240), Kampala (r= -0.28, p=0·359) and Masaka (r=-0.61, p=0·034)). However, in West Nile an increase in IPTp3+ and LLIN uptake was associated with an increase in the number of cases (r= 0.71, p=0·009 and r= 0.5, p=0·097 respectively). Efforts to increase IPTp3+ and LLIN provision at ANC likely reduce cases of MIP. Behaviour change communication to drive early and adequate ANC coverage, quality provision of IPTp3+ including directly observed therapy, LLIN use and care, as well as strong supply chain and data systems should be maintained and scaled up.

771

SUB-OPTIMAL INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY ADMINISTRATION INCREASES THE RISK OF SUBMICROSCOPIC *PLASMODIUM FALCIPARUM* INFECTION IN PREGNANT WOMEN: A PRECONCEPTION COHORT STUDY IN BENIN

Cornélia Priscile Hounkonnou[1], Nicaise Tuikue Ndam[1], Nadine Fievet[1], Manfred Accrombessi[2], Emmanuel Yovo[2], Atikatou Mama[2], Darius Sossou[2], Bertin Vianou[2], Achille Massougbodji[2], Valérie Briand[3], Michel Cot[1], Gilles Cottrell[1]

[1]*Institut de Recherche pour le Développement, Paris, France,* [2]*Institut de Recherche Clinique du Bénin, Abomey-Calavi, Benin,* [3]*Institut National de la Santé et de la Recherche Médicale, Bordeaux, France*

Malaria in pregnancy (MiP) contributes to harmful maternal and neonatal health consequences. The MiP prevention is mainly provided by intermittent preventive treatment with sulfadoxine-pyrimethamine (IPTp-SP), recommended in sub-Saharan Africa by the World Health Organization (WHO) but is contraindicated in the first trimester. A high heterogeneity of the IPTp-SP implementation (often sub-optimal) in terms of number of doses and timing is observed in the field. For the first time, we assessed the impact of this heterogeneity on MiP, mainly on submicroscopic infections. Using data from a cohort study conducted in Benin from 2014 to 2017, we followed-up 273 Beninese women from preconception to delivery. Data were collected about IPTp intake, a monthly malaria screening and a precise dating of the gestational age (GA) were performed. We investigated the effect of IPTp intake on the number of *P. falciparum* infections after 17 weeks of gestation using a negative binomial model. The proportion of women who had at least two IPTp intakes during pregnancy was 77.3%. The median GA at the first IPTp intake was 22 weeks of gestation (wg). A late first IPTp intake (>21 wg) was positively correlated with an increased number of *P. falciparum* infections (adjusted incidence rate ratio=1.34; p=0.098). The number of IPTp intakes was not associated with the number of submicroscopic infections (adjusted incidence rate ratio=1.22, p=0.543). The late first IPTp intake provides a sub-optimal protection against *P. falciparum* infections, especially submicroscopic infections. This highlights the need for a new IPTp drug that will start in early pregnancy.

772

SULFADOXINE-PYRIMETHAMINE TREATMENTS IN PREGNANT WOMEN: IN VIVO EFFICACY AND PREVALENCE OF RESISTANCE MARKERS IN OUELESSEBOUGOU, MALI

Almahamoudou Mahamar[1], Gaoussou Santara[1], Sidi Mohamed Niambele[1], Sekouba Keita[1], Bacary S. Diarra[1], Adama Dembele[1], Seydou Traore[1], Mamoudou Samassekou[1], Oumar M. Dicko[1], Ibrahim H. Soumbounou[1], Moussa Traore[1], Oumar Attaher[1], Djibrilla Issiaka[1], Halimatou Diawara[1], Patrick E. Duffy[2], Michal Fried[2], Alassane Dicko[1]

[1]*Malaria Research and Training Center (MRTC), Bamako, Mali,* [2]*Laboratory of Malaria Immunology and Vaccinology (LMIV), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, MD, United States*

Placental malaria is associated with poor outcomes for pregnant women and their babies. To improve pregnancy outcomes, WHO recommends monthly anti-malarial treatment with sulfadoxine pyrimethamine (SP) after the first trimester as well as use of insecticide-treated bed nets throughout pregnancy. Continuous monitoring of resistance to SP is needed. A cross-sectional study of pregnant women was conducted at the peak of transmission season in October 2019. Pregnant women (PW) were randomly selected in the community based on the population census. Blood smears (BS) and dried blood spot samples were collected for all participants at baseline. Participants who presented with malaria symptoms were assessed by Rapid Diagnostic Test (RDT) for *P. falciparum* infection and those with positive results received standard antimalarial treatment with artemether-lumefantrine. Participants without symptoms

received SP as intermittent preventive treatment (IPTp-SP) so long as a month had passed since their last IPTp-SP treatments. Full clinical and parasitological evaluations were performed on days 2, 3, 7, 14, 21 and 28 after receiving SP dose. Of 321 PW screened, 60.7% (195/321) had not received IPTp-SP the last month, and 36 of the 195 PW (18.5%) had asymptomatic infections and were analyzed for this study. All participants were free of parasites at day 7. Treatment failure occurred in 5.5% of the participants (2/36), one on day 14 and one on day 28. Analyses of molecular markers associated with *P. falciparum* resistance to SP as well as genotyping to distinguish recrudescence versus reinfections are ongoing. In this study area, in-vivo treatment efficacy of SP-IPTp by day 14 and 28 was observed in 34/36 and 35/36 pregnant women respectively. SP-IPTp remains a viable strategy to prevent placental malaria. .

773

ACROSS-BORDER *PLASMODIUM VIVAX* OUTBREAK IN AMAZONIAN GOLD MINING CONTEXT; AMERINDIAN COMMUNITY ENGAGEMENT FOR CONTROL

Helene Hiwat[1], Hedley Cairo[1], Loretta Hardjopawiro[1], Dayanand Panchoe[2], Malti Adhin[2], Edward Van Eer[2], Oscar Mesones[3], Stephen Vreden[2]

[1]*Ministry of health Malaria Program Suriname, Paramaribo, Suriname,* [2]*National Malaria Elimination Task Force Suriname, Paramaribo, Suriname,* [3]*PAHO Suriname, Paramaribo, Suriname*

Suriname and French Guiana have gold mining sites along their shared border river. These are mostly small-scale illegal mines worked by migrant miners. The Wayana –Amerindian- tribe has villages on both sides of the border. The Wayana provide boat services and goods to the miners. Malaria transmission had been interrupted in the Wayana villages in Suriname since 2016. Information sharing between countries revealed a *Plasmodium vivax* (Pv) outbreak at the border. Between Oct-4 and Nov-11, 2018, 12 cases were reported among which 3 young children. Case investigation indicated that the outbreak originated in an illegal French mining site. Due to policy and regulations French authorities do not provide health services in illegal mining sites. In turn the mostly undocumented miners are reluctant to access French health services in fear of being apprehended by authorities. These barriers do not exist in Suriname. French interventions were limited to active case detection (ACD) in the villages, and diagnosis and treatment at local clinics. A small number of mosquito nets were made available at the clinics. Radical treatment of Pv with primaquine (PQ) in French Guiana is restricted to people with known G6PD status. G6PD testing is not done on site. In Suriname, ACD and mosquito net distribution was done in village and mining communities. Health providers organized an information meeting with the tribal chief and Surinamese and French village elders. Support was obtained for the distribution of mosquito nets by the Wayana chief in the French villages. Also, French village elders were informed that their people, if infected, could obtain complete treatment (including PQ) in Suriname. Malaria microscopy capacity in the village and Malaria services in the mining communities were strengthened. In addition, the location was temporarily included in a pilot study in which self-diagnosis and self-treatment kits are provided at the border to miners working in French Guiana (MALAKIT). A total of 31 cases from this area were diagnosed in Suriname during the outbreak. Local transmission has again been interrupted since December 2018.

774

OPERATIONALIZING MALARIA ELIMINATION: EXPERIENCE FROM A PILOT MALARIA ELIMINATION PROJECT IN TOUNGUP TOWNSHIP, RAKHINE OF MYANMAR

Kyaw M. Tun[1], Aung Thi[2], Thant Z. Aung[3], Hein H. Nyan[3], Ei Ei W. Aung[3], Ye Thu Aung[3], Khin T. Win[1], Ye Hein Naing[1], Saw Lwin[1], Phyoe Yarzar[1]

[1]*University Research Co., LLC, Yangon, Myanmar,* [2]*National Malaria Control Programme, Ministry of Health and Sports, Myanmar, Nay Pyi Taw, Myanmar,* [3]*University Research Co., LLC, Toungup, Myanmar*

In accordance with the World Health Organization's Global Technical Strategy (GTS) for malaria elimination (2016-2030), the National Malaria Control Program (NMCP) in Myanmar initiated in 2018 pilot malaria elimination activities in Toungup township of Rakhine State with support from the PMI/USAID Defeat Malaria Project. Desk review and key informant interviews were carried out to identify key elements to strengthen the malaria surveillance system. Defeat Malaria together with the NMCP and other relevant stakeholders, developed a malaria elimination model in line with the GTS. The development of a malaria elimination plan, standard operating procedures and tools created a capacity development package for basic health services (BHS), hospital staff, village malaria workers (VMW), and general practitioners (GP). Establishment of a Township Malaria Elimination Coordination Committee promoted inter-sectoral coordination and enhanced community involvement. Mapping of malaria implementation partners ensured universal provision of malaria services. In 2019, 38,383 long-lasting insecticidal nets (LLINs) were provided to 76,445 persons (1.99 people per LLIN). To cover 240 villages/wards, 216 surveillance agents (BHS, GP, VMW) were mobilized for early detection of cases and timely notification. Out of 240 reporting units, 227 villages (95% of all villages) reached the desirable level of blood testing targeting ≥7% for annual blood examination rates. All identified 66 malaria cases were managed according to the national treatment guidelines except for 3 cases treated by the army medical assistants and mobile malaria workers who served at remote worksites. Case investigations were conducted and managed appropriately for 61 of the 63 (97%) notified malaria cases. The number of active foci was reduced from 68 in 2018 to 20 in 2019. Three persistently high residual malaria transmission areas were piloted for provision of additional measures targeted to marginalized forest goers and all three areas became free for local malaria transmission since October 2019.

776

INCREASED MALARIA CASES AND DEATHS IN BOTSWANA, WHAT NEEDS TO BE DONE TO ATTAIN ELIMINATION

Refilwe Yvonne Senyatso

Ministry of Health, Gaborone, Botswana

Botswana has made significant progress in the fight against malaria by revamping up strategies and interventions for malaria elimination. For example, in 2000, malaria cases were over 8056 with an incidence rate of 43 per 1000 population. However, in 2011 Botswana's incidence rate was under 1 per 1000 population. This was in part attributed to the following: the introduction of Artemether-Lumefantrine and the discontinuation of Sulphadoxime-Pyrimethamine; and the introduction of free mass-distribution of LLINs in addition to IRS. The country has managed to maintain this incidence rate to date. This has been supported by disaggregation of cases and utilization of Case based surveillance, as well as the use of primaquine. For a country that is determined to eliminate malaria, this alone is not sufficient; considering the number of malaria outbreaks that have occurred in recent years. For example, in 2014, 2016, 2017 and currently 2020, the country has experienced outbreaks that also resulted in significant mortalities. Fatality rates stood at 1.63% in 2014, 1.71% in 2018 and over 2.5% in 2019. Among the factors that have been responsible for such upsurges include: suboptimal IRS coverage in vulnerable communities, leaving majority susceptible to malaria; inadequate capacity for carrying out entomological surveillance

essential for guiding the appropriate implementation of vector control interventions; communities' apathy towards malaria as a disease of public health significance; the decline in malaria cases resulted in clinicians' low index of suspecting malaria in patients presenting with fever or other malaria symptoms. These factors, combined with the mismatch between financial support and the expected resources needed to achieve malaria elimination as well as the inadequate health system have led to difficulty in achieving malaria elimination vision. There is therefore a need to reorient and elevate malaria as a priority disease. This requires increased resources which include human, financial and infrastructural capabilities at all levels. Achieving this will enable the country to attain malaria elimination - a goal set in 2011.

777

UNDERSTANDING WHERE AND WHY THEY SEEK CARE FOR FEBRILE ILLNESS FINDINGS FROM FOREST GOERS IN MALARIA HOTSPOT TOWNSHIPS OF MYANMAR

May Me Thet, Naw Eh Thi Paw, Myat Noe Thiri Khaing, Su Su Zin, Sandar Oo, Si Thu Thein

Population Services International Myanmar, Yangon, Myanmar

Forest goers are bearing high malaria infection risk due to their job nature in forests. While moving towards elimination in Myanmar, they become the target group as understanding their care seeking behaviors would enable programmers design tailored interventions. The study aims to explore how these populations seek care for fever usually, during their last fever episode and reasons around decision-making. A mixed-methods study, a household survey and 24 in-depth interviews (IDI), was conducted. Household survey applied two-staged cluster sampling and households with at least one forest goer were recruited. Data on sources of care for fever and malaria Rapid Diagnostic testing (RDT) in different scenarios; in village and in forest and last episode of fever were collected. Of 510 respondents, 62.8% sought care outside for fever usually. Among them, 69.8% consulted community health volunteers (CHV) and 13.3% did government basic health staff (BHS) within their vicinity if they suffered fever in village. For forest fever cases, 96.6% went back to their neighborhood providers. If they chose providers located outside for both scenarios, top choices were BHS and private facilities. Similar sources were reported for usual RDT testing. During their last fever episode, 66.4% consulted a provider. If it was in village, top source was their neighborhood CHV (60%) and if it was in forest, top providers were neighborhood CHV (63.6%) and BHS (18.2%). The top sources outside for both scenarios were facilities. Only 24.6% took RDT and sources were similar as fever. Preferences for neighborhood CHV and BHS were explained by convenience, trust, long-term relationship and flexible payment. If these providers were not available or disease worsen after first treatment, they would go the second provider outside their neighborhood and facilities. The study highlighted the role of CHV and that malaria diagnosis and treatment within 24hr might not always be possible because of forest goers' preference for neighborhood providers. Therefore, it is critical that these CHVs are equipped with services that could facilitate prompt and proper care seeking of forest goers.

778

EFFECTIVENESS OF THAILAND'S 1-3-7 MALARIA SURVEILLANCE STRATEGY TO REACH ELIMINATION BY 2024

Prayuth Sudathip[1], Sathapana Naowarat[2], Suravadee Kitchakarn[1], Jui A. Shah[2], Felicity Young[2], Deyer Gopinath[3], Maria Dorina Bustos[3], Niparueradee Pinyajeerapat[4], Cheewanan Lertpiriyasuwat[1]

[1]*Division of Vector Borne Diseases, Ministry of Public Health, Nonthaburi, Thailand,* [2]*Inform Asia: USAID's Health Research Program, RTI International, Bangkok, Thailand,* [3]*World Health Organization, Bangkok, Thailand,* [4]*Regional Development Mission for Asia, United States Agency for International Development, Bangkok, Thailand*

Thailand's strong malaria elimination program relies on effective implementation of its 1-3-7 surveillance strategy, which was endorsed and implemented nationwide in 2016. For each confirmed malaria patient, the Ministry of Public Health's Division of Vector Borne Diseases (DVBD) ensures case notification within 1 day, case investigation within 3 days, and foci investigation within 7 days. Together with the DVBD and the US President's Malaria Initiative, Inform Asia: USAID's Health Research Program conducted an auto-regressive integrated moving average (ARIMA) time series analysis with seasonal decomposition to assess the effect of the 1-3-7 strategy implementation on malaria incidence. The analysis included all confirmed malaria cases from public health facilities for fiscal years 2012 to 2019 (n = 33,835), divided into high- and low-prevalence areas based on a threshold of 100 annual cases at the start of the study period. Prior to the 1-3-7 strategy's launch, malaria incidence was 171.19 and 2.84 cases per 100,000 population among high- and low-prevalence provinces, with incidence decreasing quarterly by 1.23 and 0.02, respectively. The ARIMA model showed a statistically significant increasing decline in the first year after the launch, with quarterly incidence decreasing by 1.26 and 0.10 ($p<0.05$) respectively. Each subsequent year saw further reductions, averaging 2.45 and 0.17 ($p<0.05$) per quarter. Comparative t-tests showed that timeliness improved significantly ($p<0.05$) for each key component in the first 3 years of implementation—18% to 74% for case notification, 39% to 81% for case investigation, and 32% to 72% for foci investigation. Although the ARIMA model does not account for other malaria interventions, this analysis suggests that the DVBD's 1-3-7 strategy is making substantial gains toward its malaria elimination goal of 2024. Next steps could model potential enhancements to the strategy or add a non-linear model to capture the gradual nature of improvements in malaria elimination programming. Thailand's 1-3-7 strategy is a useful example for other Greater Mekong Subregion countries aiming to eliminate malaria.

779

WORKING TOWARD MALARIA ELIMINATION: RESULTS FROM THAILAND'S SUBNATIONAL VERIFICATION PROGRAM

Cheewanan Lertpiriyasuwat[1], Praparat Promeiang[1], Suravadee Kitchakarn[1], Prayuth Sudathip[1], Jerdsuda Kanchanasuwan[1], Tannikar Toangard[1], Sathapana Naowarat[2], Felicity Young[2], Deyer Gopinath[3], Niparueradee Pinyajeerapat[4], Jui A. Shah[2]

[1]*Division of Vector Borne Diseases, Department of Disease Control, Ministry of Public Health, Nonthaburi, Thailand,* [2]*Inform Asia: USAID's Health Research Program, RTI International, Bangkok, Thailand,* [3]*World Health Organization, Bangkok, Thailand,* [4]*Regional Development Mission for Asia, United States Agency for International Development, Bangkok, Thailand*

In line with its goal to eliminate malaria by 2024, Thailand's Division of Vector Borne Disease (DVBD) has launched a rigorous subnational verification program to recognize provinces that have interrupted local malaria transmission. The program's first year resulted in recognizing 35 of 77 provinces as malaria free at the 2019 national World Malaria Day event. The DVBD channeled lessons learned into a revised program for 2020, using a suite of verification tools, including a manual and self-assessment checklist, to prepare Provincial Health Offices (PHOs). These country-specific tools build on the World Health Organization's (WHO's) requirements for national certification and assess five realms: surveillance system; diagnosis and treatment; a disease prevention and control committee; planning, monitoring, and evaluation; and cross-partner resource mobilization. This year, Inform Asia: USAID's Health Research Program accompanied the DVBD to Satun province to document the verification of the PHO's work. The PHO presented its malaria situation and activities that interrupted transmission. The DVBD staff then validated the absence of locally transmitted cases, reviewing surveillance data from the last four years. Satun recorded 18 cases between fiscal years 2015 and

2018 in the malaria surveillance system. For each case, the DVBD reviewed facility registers and case investigation documentation to confirm proper classification as "imported." Each case was also cross-referenced with the general health reporting system. The DVBD found an additional 7 potential cases from the health reporting system over the same time period. Upon further investigation, these 7 patients were determined to be suspect malaria cases who received differential final diagnosis. In 2021, the DVBD will closely monitor the verification process, including adequacy of current tools to support PHOs. Refining a systematic and reliable subnational verification process supports Thailand's goal of applying for WHO national-level certification by 2024. The process may also be useful for other Greater Mekong Subregion countries that pursue malaria elimination.

780

NATURAL INFECTIONS WITH DIFFERENT *PLASMODIUM* SPECIES INDUCE ANTIBODIES REACTIVE TO A CHIMERIC *PLASMODIUM VIVAX* RECOMBINANT PROTEIN

Jessica McCaffery[1], Balwan Singh[2], Doug Nace[2], Alberto Moreno[1], Venkatachalam Udhayakumar[2], **Eric Rogier**[2]

[1]*Emory University Vaccine Center, Atlanta, GA, United States,* [2]*Centers for Disease Control and Prevention, Atlanta, GA, United States*

As prevalence decreases in a setting for any of the four human malarias, it becomes increasingly difficult to identify areas of active and ongoing transmission due to fewer persons seeking treatment. Serological assays to determine anti-malarial antibody carriage in a populace provide estimates for population-level exposure which can inform targeting and elimination campaigns. Here we employ a multiplex immunoassay to investigate IgG capture capacity of a chimeric *Plasmodium vivax* MSP1 protein (PvRMC-MSP1) initially designed to be broadly immunogenic for use in vaccine studies. The PvRMC-MSP1 antigen was investigated for its ability to act as a pan-malaria serological tool with the ability to capture IgG induced during known *Plasmodium* exposure. A set of 236 naturally exposed US traveler samples were utilized with known active infection status to: *P. falciparum* (n=181), *P. vivax* (n=38), *P. ovale* (n=13), *P. malariae* (n=4). The multiplex IgG detection assay included PvRMC-MSP1 for IgG capture as well as the four MSP1-19kD isoforms for the four human malaria species. As expected, infection with each *Plasmodium* species induced IgG against that species' MSP1-19kD antigen. Regardless of infecting *Plasmodium* species, we observed that a majority of plasma from individuals with PCR confirmed malaria infection provided a high IgG signal to the PvRMC-MSP1 chimeric protein. We observed higher assay signals for the PvRMC-MSP1 chimera vs the recombinant PvMSP1 for 89.5% (34/38) of *P. vivax*-infected individuals. As opposed to utilizing only genome-encoded antigen sequences for malaria serology, these results support further study of engineered antigens designed for increasing sensitivity or broadening anti-malarial antibody binding capacity. Future work will evaluate ability of PvRMC-MSP1 to capture anti-malaria IgG at a population level in seroepidemiological surveys.

781

APPLICATION OF SEROLOGY IN EVALUATING TWO REACTIVE RESPONSE INTERVENTIONS FOR MALARIA ELIMINATION: RESULTS FROM THE CORE COMMUNITY RANDOMIZED TRIAL IN SOUTHERN PROVINCE, ZAMBIA

Daniel J. Bridges[1], John M. Miller[1], Victor Chalwe[2], Hawela Moonga[3], Busiku Hamainza[3], Richard W. Steketee[4], Brenda Mambwe[1], Conceptor Mulube[1], Lindsey Wu[5], Kevin Tetteh[5], Chris Drakeley[5], Eric Rogier[6], Sandra Chishimba[1], Mulenga Mwenda[1], Kafula Silumbe[1], David A. Larsen[7]

[1]*PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Lusaka, Zambia,* [2]*Zambia Ministry of Health, Provincial Medical Office, Mansa, Zambia,* [3]*National Malaria Control Centre, Zambia Ministry of Health, Lusaka, Zambia,* [4]*PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Seattle, WA, United States,* [5]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [6]*Malaria Branch, Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, GA, United States,* [7]*Syracuse University Department of Public Health, Syracuse, NY, United States*

A key intervention in Zambia's success in reducing malaria incidence has been training volunteer community health workers (CHW) to test and treat suspected cases, using rapid diagnostic tests and artemether-lumefantrine (AL). CHWs also perform reactive focal test and treat (RFTAT) responses, i.e. testing everyone within a 140m radius of a confirmed index case and treating the positives with AL. To accelerate progress, we evaluated reactive focal drug administration (RFDA), i.e. presumptive treatment of individuals within 140m radius of an index case with the longer lasting dihydroartemisinin-piperaquine, against RFTAT in the two-year Community-led Responses for Elimination (CoRE) randomized controlled trial. In a subset of RFDA and RFTAT reactive responses, dried blood spots were collected, PCR tested, and positive samples genotyped with a 24-SNP barcode. Multiplex bead assays determined seropositivity in a cross-sectional endline survey of children under fifteen to 23 *P. falciparum* antigens. Overall, with ~90% of all secondary infections found in the index household, and ~80% of matched barcodes found within a household, transmission appears highly focalized to the household level. During the study, malaria incidence dropped by roughly 40% in both arms, reducing the study power to test for significant differences between the interventions. When comparing the two arms on three secondary outcomes, they were similar in terms of 1) malaria incidence using routine aggregate data, 2) reinfection rates in a longitudinal cohort, and 3) PCR positivity (<0.2 %) in the endline survey. For the primary outcome (end line serological survey), children under five in the intervention (RFDA) group were 19% (95% CI = 4–32%) and 37% (95% CI = 0–60%) less likely to test positive for long-term or short-term antigens respectively, than children under five in the control group (RFTAT), suggestive of a reduction in malaria transmission/exposure in the RFDA arm. Studies such as this demonstrate how serologic assessments may help to elucidate intervention impact in low transmission settings where traditional metrics such as parasite prevalence may be limited.

783

SUPPORTING EVIDENCE-BASED DECISION-MAKING FOR MALARIA CONTROL IN MYANMAR THROUGH ROUTINE DATA REVIEW MEETINGS

K. Mon Win[1], S. Lwin[2], M. Min Tun[3], N. Aung[4], A. Htun[4], J. Dunning[4], J. Dunn[4], S. Lavenberg[4], M. Aye[4], A. Thi[1]

[1]*Ministry of Health and Sports, Nay Pyi Taw, Myanmar,* [2]*Ministry of Health and Sports, Sagaing, Myanmar,* [3]*Ministry of Health and Sports, Ayeyarwady, Myanmar,* [4]*Clinton Health Access Initiative, Yangon, Myanmar*

Myanmar has achieved a significant reduction in malaria cases, decreasing from 182,616 cases in 2015 to 75,159 in 2018, but the burden of disease remains heterogeneous across its 13 States and Regions. As Myanmar progresses towards its elimination goals, routine review of surveillance data is critical for monitoring trends, identifying priorities, and responding to operational challenges. In consideration of this need, monthly data review meetings have been instituted at national and regional levels in Myanmar. Meetings provide a forum for National Malaria Control Program (NMCP) staff to discuss data trends, assign action items, and follow up on prior meeting priorities. We review key management, surveillance and intervention metrics to determine whether implementation of data review meetings have resulted in improvements. Data review meetings were first established in Ayeyarwady Region (0.75% of national cases in 2018), beginning in January 2019. Meetings identified townships with testing rates below established targets, monitored local transmission in remaining hotspots and travel histories for imported cases. Meetings have led to improvements in reporting completeness and timeliness. The case investigation rate has increased from 42% in 2018 to 76% in 2019 through the resolution of operational bottlenecks as discordance between reports and completed case investigations is identified and accountability is reinforced. Data review meetings have recently been extended to Sagaing Region (5.62% of national cases in 2018), and to national level. Impact of

these newly established data meetings will be evaluated over the coming year. Routine data review meetings have enabled decision-makers to use data for more effective allocation of resources and overall improvement in malaria policy, strategy and program activities. By developing a culture of data use and setting a standard for evidence-based programmatic decision-making, the meetings support stakeholders, fill data gaps, and ensure targeted interventions to increase the performance of the malaria program at all levels, accelerating progress towards malaria elimination.

784

A SCHOOL SURVEY INDICATES CONDUCIVE SETTINGS FOR MALARIA ELIMINATION IN THE HIGHLANDS PROVINCES OF PAPUA NEW GUINEA

Osama Seidahmed[1], Sharon Jamea[2], Serah Kurumop[2], Manuel Hetzel[3], William Pomat[2]

[1]*Swiss Tropical and Public Health Institute and PNG Institute of Medical Research, Goroka, Papua New Guinea,* [2]*PNG Institute of Medical Research, Goroka, Papua New Guinea,* [3]*Swiss Tropical and Public Health Institute, Basel, Switzerland*

Malaria transmission in Papua New Guinea (PNG) is mainly influenced by altitude, with low-lying areas below 1200m endemic, 1200-1600m epidemic, and higher altitudes at low risk. In anticipation of malaria elimination, a cross-sectional school survey combined with reactive case detection at the households of students was carried out in the seven Highlands Provinces of PNG between July and November 2019 to investigate local transmission. We screened a total of 5,578 students and 1,048 household members from 137 villages using malaria rapid diagnostic tests. Overall, malaria prevalence rates are low: 0.1% (range 0-0.55%) among students and 0.68% (range 0-8.06%) among household members. While no infection was detected in receptive areas below 1500m, 13 positive cases were clustered at higher altitudes, all except one at above 2000m. Nearly all the cases reported a recent trip to lower-altitude endemic areas. In addition, among all screened students and household members travelled in the last month, 23% and 40%, respectively, had reported endemic coastal provinces as their destination. The odds of malaria infections are significantly higher among surveyed participants with a travel history, 9.7-fold in the students, and 123.7-fold in the households. Use of bed nets by household members is 82.28% below 1200m, 43.96% in altitudes 1200-1600m and remarkably dropped to 17.28% above 1600m. Although coverage and effective use of long-lasting insecticidal nets decreased with altitude, the risk of local malaria remains low in the Highlands. Our findings indicate conducive settings for elimination in the Highlands of PNG with the little local transmission but the risk of importation of infections. There is an opportunity to strengthen surveillance-response systems as an intervention to control imported cases and interrupt malaria transmission in the region.

785

STRATEGY AND ACHIEVEMENTS OF THE NATIONAL MALARIA ELIMINATION PROGRAMME IN CHINASPONSE SYSTEMS FOR MALARIA ELIMINATION IN CHINA, INCLUDING VECTOR SURVEILLANCE AND CONTROL

Xiao-Nong Zhou

National Institute of Parasitic Diseases, Shanghai, China

Strategy and achievements of the national malaria elimination programme in China Jun Feng, Li Zhang, Fang Huang, Zhi-Gui Xia, Ning Xiao, Xiao-Nong ZhouNational Institute of Parasitic Diseases, China CDC

Malaria was once one of the most serious public health problems in China. However, the disease burden has sharply declined and epidemic areas have shrunk after the implementation of an integrated malaria control and elimination strategy, especially since 2000. A retrospective evaluation was performed to assess the changes in malaria epidemic patterns from 1950 to 2017 at national level. From 1950 to 2017, the occurrence of indigenous malaria has been steeply reduced, and malaria-epidemic regions have substantially shrunk, especially after the launch of the national malaria elimination programme. There were approximately 30 million malaria cases annually before 1949 with a mortality rate of 1%. A total of 5999 indigenous cases were documented from 2010 to 2016, with a drastic reduction of 99% over the 6 years (2010, n=4262; 2016, n=3). There were indigenous cases reported in 303 counties from 18 provinces in 2010, but only 3 indigenous cases were reported in 2 provinces nationwide in 2016. While in 2017, for the first time, zero indigenous cases were reported in China, and only 7 of imported cases were in individuals who died of *Plasmodium falciparum* infection. Therefore, malaria elimination in China is a country-led and country-owned endeavour. The country-own efforts were a clear national elimination strategy, supported by two systems, namely a case-based, surveillance and response system and reference laboratory system. The country-led efforts were regional and inter-sectorial collaboration as well as sustained monitoring and evaluation. However, there are still some challenges, such as the maintenance of non-transmission status, the implementation of a qualified verification and assessment system, and the management of imported cases in border areas, through regional cooperation. The findings from this study can probably help improving malaria surveillance systems in China, but also in other elimination countries.

786

MULTI-LATERAL COOPERATION ON MALARIA ELIMINATION IN CHINA-MYANMAR BORDER AREAS

Fang Huang[1], Zhi-Gui Xia[1], He Yan[1], Hong-Ning Zhou[2], Jian-Wei Xu[2], Xin-Yu Feng[1], Xiao-Nong Zhou[1]

[1]*Naitonal Institute of Parasitic Diseases, China CDC, Shanghai, China,* [2]*Yunnan Institute of Parasitic Diseases, Puer, China*

Malaria remains one of the important infectious diseases in China-Myanmar border in the Greater Mekong Subregion. There are 20 counties in Yunnan Province of China and 23 townships in Myanmar sharing the adjacent border as long as 1,997 kilometers. China will eliminate malaria by 2020 and to sustain malaria free status and prevent re-establishment beyond 2020. In Myanmar , the goal is to eliminate *Plasmodium falciparum* malaria by 2025 and to eliminate malaria by 2030. The border area in both sides is outlying, hard to reach and poverty-stricken inhabited by the minority nationalities. As driven by the threaten of artemisinin resistance, WHO published «Strategy for Malaria Elimination in the Great Mekong Subregion» . In recent years, there have been several multi-lateral cooperation on malaria elimination in China-Myanmar border areas which were initiated by WHO and NIPD, China CDC as WHO Collaborating Centre. The bilateral meetings malaria elimination in China-Myanmar border areas was called once every year until 2019 to develop the strategic action plan for Malaria Elimination in China-Myanmar Border (2016-2030) , operational plan of China-Myanmar Malaria Control Program and collaboration mechanism and working groups as well. The main strategies include: 1. Universal access to malaria prevention, diagnosis and treatment to reduce disease burden in moderate to high transmission areas; 2.Transforming malaria surveillance into a core intervention to accelerate toward elimination; 3. Approaches specifically tailored for mobile and migrant population besides routine interventions; 4. Capacity building and operational research; and 5. Strengthening the enabling environment. China and Myanmar government including China CDC and Myanmar VBDC/NMCP, WHO and EHOs in Myanmar participant in this cooperation with different responsibilities to achieve the ultimate goal of malaria elimination in this region.

787

SUCCESSFUL CASE STUDIES ON MALARIA ELIMINATION WITH MULTI-PROVINCE COOPERATION IN CHINA

Ying Liu

Henan Provincial Center for Disease Control and Prevention, Zhenzhou, China

Successful Case studies on malaria elimination with multi-province cooperation in China Hong-Wei Zhang[1], Ying Liu[1], Xiao-Nong Zhou[2] (1. Henan provincial CDC, China; 2. National Institute of Parasitic Diseases, China CDC)

Vivax malaria was historically epidemic in Huang-Huai plain of China and *Anopheles sinensis* was the main vector. There were two high epidemics with malaria cases accounted for 93.1% and 91.2% of total cases of whole country in 1960 and 1970, respectively. Considerable success had been achieved and vivax malaria cases have been reduced significantly in the Huang-Huai plain in the end of 1980s, and malaria incidence was below 1/10000 in most areas. However, the incidence of vivax malaria had resurgence early 21th century and malaria outbreaks were found in 2006. This case study was undertaken on patterns of vivax malaria outbreak in Huang-Huai plain in 2006, and how to take the quick response to cut down the outbreak by MDA and case management. In 2006, malaria cases were mainly reported during August and November, 8681 cases were reported, accounted for 89.7% of total cases found in outbreak spots. Monthly *P. vivax* malaria incidence showed a seasonal pattern, whose peak period was from July to November, a period when nearly 86.58% of total malaria cases were reported in Yongcheng county. The re-emergence was effectively controlled by an extensive control strategy on patient management supported by central government and Global Fund. Malaria incidence dropped dramatically in Huang-Huai plain after 2007. A total of 769 vivax malaria cases were reported in 2011. Only 30 local vivax malaria cases were reported in Anhui province, and no local transmission vivax malaria was reported in Henan, Jiangsu, and Shandong provinces in 2012. Particularly due to the special ecological characteristics in Huang-Huai plain, the intervention of vector control is not implemented. Finally, the challenges in elimination of malaria in this region are highlighted. The authors suggested that strategies to understand and address these phenomena are needed urgently if the global elimination of malaria is to succeed.

788

FROM *PLASMODIUM VIVAX* OUTBREAKS TO ELIMINATION: LESSONS LEARNED FROM RETROSPECTIVE STUDIES AND MODELLING

Guo-Jing Yang[1], Xiao-Nong Zhou[2], Hong-Wei Zhang[3], Ying Liu[3], Monica Golumbeanu[1], Nakul Chitnis[1], Michael White[4], Tom Smith[1], Melissa A. Penny[1]

[1]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [2]*National Institute of Parasitic Diseases, Shanghai, China,* [3]*Henan CDC, Zhengzhou, China,* [4]*Institute of Pasteur, Paris, France*

Malaria was once a serious public health problem in China. Following implementation of integrated malaria control and elimination programs, the disease burden has sharply declined, with no indigenous cases announced in 2017 at national level. To thoroughly understand the malaria transmission patterns and epidemic characteristics and further provide new insights for guiding *P. vivax* malaria control and elimination, retrospective studies were carried out. Historical data from a pilot study in Guantang, Luyi, carried out in central China from 1971-1995, was digitized. These data were used to parameterize an existing compartmental model of *P. vivax* dynamics. Human *P. vivax* inoculation studies in provinces of Henan, Hunan, Yunnan, Guizhou and Guangxi in 1980s were collated to understand and estimate the types of incubation periods and relapse times of *P. vivax* in China. After 25 years of continuous integrated malaria control activities, the incidence of malaria in Guantang decreased from 4333 cases per 10,000 in 1970 before integrated implementation to 0.23 cases per 10,000 in 1991, with no reported cases in 1992-1995. Both short- and long-incubation period phenotypes of *P. vivax* were observed in various regions in China. Mathematical models supported the retrospective findings that the implementation of mass campaign of liver stage hypnozoite eradication played an important role on the pathway towards national malaria elimination. In conclusion, the integrated malaria control approach in Guantang proved to effectively control malaria and achieve elimination. Analysis of the effectiveness of different components of the program can provide guidance to other regions or countries with similar ecological settings that are aiming to move from malaria control to elimination. Challenges remain in the maintenance of non-transmission status owing to the long dormancy of liver stage hypnozoites. These observations have important implications for the assessment of radical treatment efficacy and for guiding malaria control and elimination reform.

789

LEVELS OF *PLASMODIUM FALCIPARUM* PARASITEMIA DETECTED THROUGH REACTIVE CASE DETECTION IN A LOW MALARIA PREVALENCE SETTING OF SOUTHERN PROVINCE, ZAMBIA: IMPLICATIONS FOR SURVEILLANCE

Michael Musonda

Macha Research Trust, Choma, Zambia

Malaria caused by *Plasmodium falciparum* remains a public health problem in Zambia. However, National Malaria Indicator Surveys showed a large reduction in malaria prevalence from 2010 to 2018 in children under five years in Southern Province. Reactive case detection using rapid diagnostic tests (RDTs) is widely practiced for malaria surveillance and control in this region. However, successful malaria elimination strategies require understanding the distribution of levels of parasitemia within the population to assess the sensitivity of RDTs for reactive case detection. Levels of *P. falciparum* parasitemia were measured from July 2015 to August 2018 in the catchment area of Macha, Choma District, Southern Province, Zambia. Health workers from 14 rural health centres reported all index cases to Macha Research Trust (MRT). An MRT study team visited index case and neighbouring households within 250 meters, obtained informed consent, administered questionnaires, performed an RDT, and collected a dried blood spot (DBS) for detection of *P. falciparum* DNA by quantitative polymerase chain reaction (qPCR; limit of detection 1 parasite/μL of blood). A total of 7,619 participants were enrolled, of which 237 were positive by qPCR, with parasite counts ranging from 1 to 72,392 parasites/μL. Assuming an RDT detection threshold of 100 parasites/μL, 186 (78%) parasitemic individuals were below this theoretical threshold. Median parasite counts above and below the theoretical threshold were 900 and 6 parasites/μL, respectively. In fact, RDTs were positive in 27 (15%) individuals with levels of parasitemia below 100 parasite/μL and in 30 (59%) individuals with parasitemia above 100 parasite/μL. Participants between 6 to 15 years were more likely to be RDT positive and had higher levels of parasitemia. These findings suggest that most *P. falciparum* infections in this low malaria transmission setting are below the detection limit of standard RDTs, and school-age children may be parasite reservoirs for local malaria transmission.

790

MALARIA ELIMINATION SURVEILLANCE SUCCESSES AND CHALLENGES IN BOTSWANA

Erica Berlin[1], Refilwe Senyatso[2], Mpho Mogojwe[2], Setshwano Gaosenkwe[1], Davies Sedisa Ntebela[2], Solomon Gobezie Worku[1], Tjantilili Mosweunyane[1]

[1]*Clinton Health Access Initiative, Gaborone, Botswana,* [2]*Ministry of Health, National Malaria Programme, Gaborone, Botswana*

Botswana reoriented its malaria program towards elimination in 2010 due to a drastic reduction in confirmed malaria cases from 8056 in 2000 to 885 in 2009. The country has been recording incidence of less than 1 per 1000 population since 2011, reaching a low of 0.1 per 1000 population in 2019. Health facilities report weekly on aggregated indicators for

priority diseases, including malaria, through the Integrated Disease Surveillance Response (IDSR) system. Since 2012, malaria cases have also been notified via a case-based surveillance (CBS) system that collects key data points from each confirmed malaria case. A surveillance assessment in 2015 found that Botswana's CBS system had low completeness, timeliness, and data quality, and that the existing paper and Excel-based systems made it difficult to use data for good decision making because data were not available in real time. As a result of recommendations from the assessment, a malaria real time reporting system using DHIS2 Tracker Capture (DHIS2) was rolled out in 2017. Data from 2012-16 were compared to downloaded CBS data from the malaria DHIS2 system collected in 2018. The analysis found that completeness of case residence (69% vs. 100%), case classification (93% vs. 97%) and travel history (68% vs. 98%) all increased statistically significantly at the p<0.001 level between baseline and 2018, the year after DHIS2 was rolled out. The difference between the IDSR and CBS systems dropped from 40% in 2012-16 to under 5% in 2018. Data audits, support, and mentorship visits post initial roll-out gave opportunities for refresher skills building, troubleshooting, entering missing data, and data analysis. This also gave opportunity for technical IT support. Challenges identified at these meetings included initial system bugs, suboptimal retention of knowledge from initial trainings and unclear roles and responsibilities among staff at district level. The findings from this analysis indicate that the rollout of DHIS2 improved completeness of CBS data enough to accurately inform targeting of elimination interventions, but continuous training and system improvements are needed to maintain gains.

791

ASSESSMENT OF COMMUNITY HEALTH WORKER ADHERENCE TO MALARIA TREATMENT GUIDELINES IN NAMIBIA

Jerobeam Hamunyela[1], Petrina Uusiku[1], Stark Katokele[1], Dennis Walusimbi[2], Eliza Walwyn-Jones[3]

[1]*National Vector Borne Disease Programme, Windhoek, Namibia,* [2]*Clinton Health Access Initiative, Windhoek, Namibia,* [3]*Clinton Health Access Initiative, Gaborone, Botswana*

As part of efforts to eliminate malaria in Namibia, the National Vector Borne Disease Programme (NVDCP) has been scaling up community case management (CCM) since 2017. 200 Government Community Health Workers (CHWs) and 124 partner funded CHWs are providing malaria services in the community to combat access to care challenges in sparsely populated regions of the country. To evaluate the quality of care provided by these cadres, the NVDCP tested CHW performance throughout trainings and post deployment, alongside review of key operational factors potentially influencing adherence to guidelines. In 2019, the NVDCP carried out field visits to test CHWs on knowledge retention in four field surveys and interviewed CHWs, supervisors, and community members to collect data on CHW performance, acceptability and challenges. CHWs performance on pre and post theoretical and practical assessments were collected and cut offs were defined to group these marks in to fail, pass and high pass categories. Results from a paired t-test indicated a significant improvement in test scores post training, from a mean score of 54.9% (95% CI 62.0-57.8) pre- test to 86.1% (95% CI 84.9-87.4) post-test (p<0.001). Retention of knowledge from trainings was high, with all CHWs (N=64) tested in the field passing RDT observations and 62/64 (97%) passing on treatment scenarios. Key challenges identified included low availability of waste disposal bags, phone credit for reporting and stock cards and frequency of supervision (only 62% supervisions were reported to occur at recommended frequency). This evaluation indicates that trainings for CHWs in Namibia are impactful, and knowledge on appropriate case management practices are well retained. However, the surveys highlighted a need to address operational factors that will impact CHWs' ability to adhere to guidelines on waste disposal, reporting and stock management. This evaluation represents the first attempt to assess the CHW programme in Namibia, to be supplemented going forwards with routine data collection and review. The NVDCP will use these results to inform quality scale up of the CHW programme.

792

BETTER LONG-TERM PROTECTION WITH A LIVER-STAGE DEFECTIVE *PLASMODIUM* THAN THE WILD TYPE IN CHEMOPROPHYLAXIS VACCINATION

Tejram Sahu, Godfree Mlambo, Ella Gehrke, Julia Romano, Yevel Flores-Garcia, Fidel P. Zavala, Isabelle Coppens

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

Chemoprophylaxis Vaccination (CVac) is promising vaccination approach against malaria. CVac provides better sterilizing immunity, with two-log less sporozoites than the Radiation Attenuated Sporozoite (RAS) vaccine. We have engineered a *Plasmodium* mutant named PbATG8-OE (*Plasmodium berghei* OverExpressing ATG8) that exhibits a severe growth defect in cultured hepatocytes, and significant delay in patency after infection with sporozoites *in vivo*, thus likely exposing liver stage antigens to the immune system longer than WT parasites. We hypothesize that PbATG8-OE parasites would be more sensitive to a chemoprophylaxis treatment, hence an ideal antigenic constituent of a CVac regimen. Comparing the vaccine potential of *P. berghei* WT (parental) and PbATG8-OE using a CVac regimen, we show that PbATG8-OE provides superior memory response (100%) than WT parasites (60%) and confers better long-term protection (40% vs. 20% for WT). PbATG8-OE-CVac generates a protective CSP specific anti-sporozoite antibody-response unlike WT-CVac. Interestingly, the protection elicited by PbATG8-OE-CVac is CD8-T cell-independent, but is mostly IFN-γ independent. PbATG8-OE-CVac primes CD8-T cells better than WT-CVac. Moreover, PbATG8-OE-CVac mice maintain antigen exposed CD8-T cells in a significantly greater proportion than WT-CVac. We are currently investigating the protective mechanism in mice infected with PbATG8-OE-CVac, which would lead to the discovery of new antigens and memory effectors and may help develop better malaria vaccine.

793

EXTENDED EFFICACY AND SAFETY OF GMZ2 CANDIDATE MALARIA VACCINE IN A PHASE IIB, RANDOMIZED, CONTROLLED, DOUBLE-BLIND, MULTI-CENTRE TRIAL IN MALARIA EXPOSED CHILDREN

Sylvester D. Dassah[1], Bright Adu[2], Sodiomon B. Sirima[3], Benjamin Mordmüller[4], Ulysse Ateba Ngoa[5], Frank Atuguba[1], Fareed K. N. Arthur[6], Mark Kaddumukasa[7], Clare Flach[8], Peter Bang[9], Peter G. Kremsner[10], Paul Milligan[8], Michael Theisen[9]

[1]*Navrongo Health Research Centre, Navrongo, Ghana,* [2]*Department of Immunology, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana,* [3]*National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso,* [4]*of Tropical Medicine, University of Tübingen, Tübingen, Germany,* [5]*Centre de Recherches Médicales de Lambaréné, Lambaréné, Gabon,* [6]*Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana,* [7]*Makerere University College of Health Sciences, Kampala, Uganda,* [8]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [9]*Department for Congenital Disorders, Statens Serum Institut, Copenhagen, Denmark,* [10]*Institute of Tropical Medicine, University of Tübingen, Tübingen, Germany*

GMZ2/alum candidate malaria vaccine was recently assessed in a phase IIb trial, involving 1849 participants 12 to 60 month of age in four countries in central, eastern and western Africa. During the first 6 months of follow-up, GMZ2/alum showed a vaccine efficacy (VE) of 14% (95% confidence interval [CI]: 3.6%; 23%) against clinical malaria. Here we report the extended follow up of safety and efficacy. A total of 1849 (GMZ2 = 926, rabies = 923) children aged 11 - 60 months were randomized to receive intramuscularly, either 3 doses of 100µg GMZ2/alum or 3 doses of rabies vaccine as control 28 days apart. The children were passively followed up for 22 months for clinical malaria episodes. Malaria incidence was markedly seasonal at all sites. There were a total of 1,758 malaria episodes in the GMZ2/alum group and 1,813 in the rabies vaccine group after the follow-up. The according to protocol (ATP) analysis, VE adjusted for age

and site was 6.5% (95%CI: -2.0%; 14.3%). When the follow-up period was divided into 3-month intervals, VE adjusted for age and site was 6.04% (95% CI: -6.47%; 17.08%, p=0.329), 20.51% (95%CI: 4.88%; 33.58%, p=0.012), and 28.12% (95%CI: -4.72%; 50.66%, p=0.086) in the first, second and third period, respectively. VE in older children (36 to 60 months old) was 37.79% (95% CI: 18.45; 52.55%; p = 0.001) compared to 3.57% (95% CI: -22.73; 24.23%; p = 0.768) in younger children (11 to 35 months old) at the peak of efficacy. The rate of severe malaria was not significantly different between the two groups in both ATP and intention to treat analysis. There were no GMZ2 vaccine related severe adverse events recorded in the extended follow up period. GMZ2/alum was safe and well tolerated. The highest VE was observed between the third and fifteenth months of follow up in older children where it appeared to act in concert with naturally acquired immunity.

794

QUANTIFICATION OF *PLASMODIUM FALCIPARUM* VS*. P. KNOWLESI* IN RHESUS LIVER: IMPLICATIONS FOR MALARIA VACCINE STUDIES IN NON-HUMAN PRIMATE MODELS

Melanie J. Shears[1], Annette M. Seilie[1], B. Kim Lee Sim[2], Stephen L. Hoffman[2], Sean C. Murphy[1]

[1]*University of Washington, Seattle, WA, United States,* [2]*Sanaria, Rockville, MD, United States*

Rhesus macaques are recognized as one of the best models of the human immune system and are valuable pre-clinical models for malaria vaccine development. Rhesus are highly susceptible to *Plasmodium knowlesi* (Pk), a parasite that naturally infects non-human primates and causes zoonotic infections in humans. The Pk/rhesus challenge model is an established platform for evaluating malaria vaccine immunogenicity and efficacy and provides a stringent model for assessing strategies prior to human trials. An alternate platform is the *P. falciparum* (Pf)/rhesus model. As rhesus do not support Pf blood stage infection, this platform is primarily used to assess Pf vaccine immunogenicity since animals are not amenable to challenge with a blood stage endpoint. Despite this, previous studies indicate that rhesus have some susceptibility to Pf sporozoite (SPZ) infection, with PfSPZ able to infect rhesus primary hepatocytes *in vitro*, and Pf 18S rRNA detectable in the rhesus liver 3-6 days after intravenous injection of aseptic, purified PfSPZ *in vivo*. For live-attenuated sporozoite vaccines, which almost certainly rely on sporozoite invasion in the liver for antigen delivery, such findings have allowed these vaccines to be successfully evaluated in both rhesus platforms. However, we still have an incomplete understanding of rhesus susceptibility to Pf sporozoites *in vivo*, limiting our ability to directly compare data across platforms. Here we investigated and quantified the differing susceptibility of rhesus to liver stage infection with purified cryopreserved PkSPZ versus PfSPZ using the highly sensitive 18S rRNA RT-PCR assay. Whereas high dose PkSPZ sporozoite infection led to high 18S rRNA biomarker signal in the liver 5 days post-infection (9.45-10.09 log10 copies/g liver), the 18S rRNA biomarker copy number in the liver after high dose PfSPZ infection was 3-5 orders of magnitude lower (4.91-6.61 log10 copies/g liver). These data further our understanding of the Pk/rhesus and Pf/rhesus malaria models and more clearly define how rhesus can best be used to study live-attenuated sporozoite vaccines.

796

COMBINED IMMUNIZATION WITH *PLASMODIUM FALCIPARUM* SEXUAL STAGE ANTIGENS *PF*S230 AND *PF*S48/45.6C SKEWS ANTIBODY RESPONSES IN FAVOR OF *PF*S230

Tracy Sarkodie-Addo[1], Stephen Kusi[1], Caleb K. Sinclear[1], Susheel K. Singh[2], Margaret M. Frempong[3], Michael Theisen[4], Daniel Dodoo[1], Bright Adu[1]

[1]*Noguchi Memorial Institute for Medical Research, Accra, Ghana,* [2]*Centre for Medical Parasitology, University of Copenhagen, Copenhagen, Denmark,* [3]*Department of Molecular Medicine, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana,* [4]*Statens Serum Institut, Copenhagen, Denmark*

Plasmodium sexual stage antigens Pfs230 and Pfs48/45 are promising transmission-blocking (TB) vaccine candidates. Levels of antibodies against these antigens correlate with their potency in reducing oocyst numbers in mosquitoes. Thus, a better understanding of the specific vaccine-related factors that enhance antibody production will aid TB vaccine design. Here, we hypothesized that the kinetics of antibodies elicited against Pfs230.pro and Pfs48/45.6C vary between single and combined immunizations in a dose-dependent manner. A group of 5 rats was immunized separately with 1.5µg/ml each of Pfs230.pro, Pfs48/45.6C and a combination of Pfs230.pro-Pfs48/45.6C. The formulation was repeated at 6.0µg/ml dose, and a separate group of 5 rats was immunized with the alum as control. Booster shots were administered on days 21 and 42. Rats were pre-bled 8 days prior to immunization and antisera were collected on days 20, 41, 63, 102, 125 and 151 post-immunization. We found a Pfs230.pro-biased, dose-dependent, synergistic interaction between Pfs230.pro and Pfs48/45.6C at the 1.5µg dose level but not at the 6µg dose level. Modeling of the antibody kinetics showed significant differences in the antibody growth and decay rates for the different formulations. These data suggest that the different vaccine formulations triggered different B-cell activation pathways. Our results imply that TB vaccines prioritizing rapid anti-Pfs230 antibody responses will benefit from the strategy of combining low-dose Pfs230 and Pfs48/45.

797

SAFETY OF PAMVAC PLACENTAL MALARIA VACCINE CANDIDATE: RESULTS OF A PHASE IB VACCINE TRIAL IN LIFELONG MALARIA EXPOSED NULLIGRAVID WOMEN IN BENIN

Euripide F. Avokpaho[1], Yannelle A. Dossou[1], Tatiana Hountohotegbe[1], Benjamin Mordmüller[2], Diane Egger-Adam[2], Philippe Deloron[3], Nicaise T. Ndam[4], Achille Massougbodji[1], Adrian J. Luty[4], Peter G. Kremsner[2], Saadou Issifou[5]

[1]*Institut de Recherche Clinique du Bénin, Cotonou, Benin,* [2]*Institut für Tropenmedizin, Universitätsklinikum Tübingen and Deutsches Zentrum für Infektionsforschung, Tübingen, Germany,* [3]*UMR216 MERIT Mère et Enfant face aux Infections Tropicales, Institut de Recherche pour le Développement, Université Paris 5, Sorbonne Paris Cité, Paris, France,* [4]*UMR 216 MERIT Mère et Enfant face aux Infections Tropicales, Institut de Recherche pour le Développement, Université Paris 5, Sorbonne Paris Cité, Paris, France,* [5]*Fondation pour la recherche scientifique (FORS), Cotonou, Benin*

We report the Benin site safety related findings of a Phase Ib trial for a VAR2CSA based vaccine-candidate, PAMVAC extemporaneously adjuvanted with either Alhydrogel or Glucopyranosyl Lipid Adjuvant-Stable Emulsion (GLA-SE), in Healthy Lifelong Malaria-Exposed, Nulligravid Adult Women in Benin. The study took place in Benin Institute of Clinical Research from November 2016 to May 2017. Twenty-one healthy female adults aged 18 to 30 years old were enrolled and randomized to receive 50 µg PAMVAC adjuvanted either with Alhydrogel (0.43mg) or with GLA-SE (2.5mg) or placebo (NaCl 0.9%). Vaccines were administered at Day 0 (1st dose), Day 28 (2nd dose) and Day 56 (3rd dose) and follow-up was done during 8 visits and lasts 256 days. All results are reported for 1st, 2nd and 3rd vaccination respectively. In terms of immediate reactogenicity, one hour after each vaccination, the main symptoms were pain at injection site (83%, 65% and 86%), swelling (11%, 20% and 14%), tenderness (5%, 15% and 0%). In terms of local and systemic reactogenicity between day 1 and day 14, participants reported headache (24%, 37% and 23%), mylgia (20%, 0% and 12%), arthralgia (18%, 26% and 18%), fatigue (12%, 21% and 23%), fever (8%, 5% and 6%), dizziness/malaise (0%, 5% and 9%) and diarrhea (0%, 5% and 9%). As unsolicited AEs, some participants experienced abdominal pain, cough, pruritus at injection site, generalized pruritus, genital infection, sore throat, malaria, dysphagia etc. All biological parameters (Eosinophils, Alanine amino-transferase,

Aspartate amino-transferase, Creatinine, Lactico Deshydrogenase, Bilirubin) were normal except for some low levels of Hemoglobin with no clinically significant interest. All the reported AEs were of mild grade. No Serious Adverse Event (SAE) was reported. The participant's retention rate was 100% . In conclusion, the 50 µg dose of PAMVAC vaccine candidate was safe in phase 1b trial as no SAEs was reported. Further development can be considered for phase 2 and 3 trial to obtain a vaccine which will reduce the burden of maternal malaria on newborns health.

798

ENVIRONMENTAL MODIFIERS OF RTS,S/AS01 MALARIA VACCINE EFFICACY IN LILONGWE, MALAWI

Griffin J. Bell[1], Matthew S. Loop[1], Tisungane Mvalo[2], Jonathan J. Juliano[3], Innocent Mofolo[2], Portia Kamthunzi[2], Gerald Tegha[2], Marc Lievens[4], Jeffrey Bailey[5], Michael Emch[1], Irving Hoffman[3]

[1]*UNC Gillings School of Global Public Health, Chapel Hill, NC, United States,* [2]*University of North Carolina Project Malawi, Lilongwe, Malawi,* [3]*UNC School of Medicine, Chapel Hill, NC, United States,* [4]*GlaxoSmithKline (GSK), Wavre, Belgium,* [5]*Brown University, Providence, RI, United States*

RTS,S/AS01 is the first vaccine against malaria to undergo pilot implementation, beginning in 2019 and vaccinating 360,000 children per year in Malawi, Ghana, and Kenya. The four-dose vaccine is given as a primary three-dose series with a fourth dose given around 18 months later. The efficacy of RTS,S/AS01 was variable among the eleven sites participating in the 2009-2014 phase III trial (NCT00866619), possibly due to differences in transmission intensity. However, a within-site examination of environmental factors related to transmission intensity and their impacts on vaccine efficacy has yet to be conducted. We implemented the phase III RTS,S/AS01 trial at the Malawi site, which enrolled 1,578 infants (6-12 weeks) and children (5-17 months) living in the Lilongwe District in Central Malawi and followed them for 3 years between 2009 and 2014. A global positioning system survey and an ecological questionnaire were conducted to collect participant household locations and characteristics, while additional data on background malaria prevalence were obtained from a concurrent Malaria Transmission Intensity (MTI) survey. Negative binomial regression models were used to assess whether the efficacy of the vaccine varied by estimated background malaria prevalence, household roof type, or amount of nearby vegetation. Vaccine efficacy did not significantly vary by estimated malaria prevalence or by roof type. However, increased vegetation cover was associated with an increase in the efficacy of the primary RTS,S/AS01 series in the 18 months before the fourth dose and a decrease in the efficacy of the primary vaccine series in the 18 months following, if the fourth dose was not given. Vegetation cover did not alter the efficacy of the fourth dose. Vegetation coverage in this study site might be a proxy for nearness to rivers or branching, shallow wetlands called "dambos" which could serve as breeding sites for mosquitoes. This modification of the efficacy of RTS,S/AS01 by vegetation cover suggests that initial vaccine efficacy and the importance of the fourth dose varies based on ecological context.

799

LONG-TERM (4-YEAR) STABILITY STUDY OF THE PFS230D1-EPA CONJUGATE

Daming Zhu, Weili Dai, Holly McClellan, Dennis Braden, Alec Allee-Munoz, Karine Reiter, Katerina Christodoulides, Lynn Lambert, Emma Barnafo, Kelly M. Rausch, David L. Narum, Patrick E. Duffy

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

Pfs230, a *Plasmodium falciparum* sexual stage protein, is the target of the most advanced malaria transmission-blocking vaccine candidate, Pfs230D1-EPA. Pfs230 is expressed in gametocytes in the human host and displayed on the surface of gametes in the mosquito host. To increase immunogenicity, the recombinant *Pichia pastoris* expressed domain 1 of Pfs230 (Pfs230D1M) was conjugated to the recombinant, nontoxic *Pseudomonas aeruginosa* ExoProtein A (rEPA) in conformance with current good manufacturing practices (cGMP). To assess the stability of this vaccine candidate during storage, the cGMP Drug Product Intermediate (DPI) Pfs230D1-EPA conjugate was stored at -80°C ± 10°C and quality control testing was conducted annually for 4 years. The stability of the vaccine candidate was evaluated by appearance, endotoxin levels, sterility, pH, protein content by UV Spec (A_{280}), and SDS-PAGE with silver staining (migration pattern). Protein immunogenicity was determined by Western blot using conformation-dependent and transmission-blocking anti-Pfs230D1 monoclonal antibodies 4F12 and 5H1 and an anti-exotoxin A polyclonal antibody. The Pfs230D1-EPA was further characterized by reversed-phase HPLC (RP-HPLC) and size exclusion chromatography with inline multi-angle light scattering (SEC-MALS). The vaccine potency was evaluated in mice at 6-month intervals. Our results showed that no alterations in physiochemical and immunological properties were detectable in long-term stored Pfs230D1-EPA conjugate. These studies demonstrate that the Pfs230D1-EPA DPI is stable for up to 4 years and has maintained its quality characteristics while in storage at -80°C ± 10°C during the entire clinical trial period.

800

ACCEPTABILITY OF MALARIA VACCINE IN CONTEXT OF PILOT INTRODUCTION IN WESTERN KENYA: RESULTS FROM THE BASELINE HOUSEHOLD SURVEY

Nelli Westercamp[1], Dorcas Akach[2], Brian Seda[2], Irene Okanda[2], Elizabeth Marube[2], Isabella Nyangau[2], Titus Kwambai[1], Simon Kariuki[2], Aaron Samuels[1]

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

Following a recommendation by the World Health Organization, the RTS,S/AS01 malaria vaccine was introduced in pilot implementation by the Ministry of Health in western Kenya in September, 2019. Due to a dosing schedule that requires new vaccine visits, the ability to reach high vaccine coverage is a concern. Vaccine uptake may also be affected by concerns about its partial protection (39% reduction of clinical malaria with 4 doses). We evaluated the acceptability of malaria vaccine to caregivers of eligible children in a random household survey conducted in July-October 2019, during the period leading up to vaccine introduction. The survey was designed to obtain a representative sample of households with children aged 5-48 months in the introduction area (46 sub-counties). We interviewed 4,169 caregivers of 4,952 children about malaria, sources of information on malaria vaccine, and willingness to vaccinate their children against malaria. Most thought of malaria as a serious concern (80.6%), common in their community (78.2%), and mostly affecting children (85.9%). Over a third (36.3%) had heard of the malaria vaccine, citing radio (37.0%), survey staff (25.1%), and public events (14.4%) as information sources. Among those who knew of the vaccine, 93.1% believed that it was beneficial, 88.0% safe, and 89.4% that it provided some protection. Before assessing acceptability, we provided a brief overview of the malaria vaccine to all caregivers, regardless of prior awareness of the vaccine. When asked "would you accept the new malaria vaccine for your child", 99% agreed for each of these conditions: if it was offered as part of routine vaccination; if it was safe; free; and available; and 99% said they would return for each of the 4 doses. Acceptability remained high (98.3%) even when reminded that children may still get malaria after vaccination. Few (3.3%) who were hesitant cited concerns over efficacy, side effects, or time constraints. Overall, baseline conditional acceptability of malaria vaccine among caregivers in western Kenya was high. Future vaccine coverage surveys may indicate whether these beliefs translate into vaccine uptake.

801

T CELL RESPONSES FOLLOWING IMMUNIZATION WITH THE PAMVAC VACCINE CANDIDATE FOR PLACENTAL MALARIA DURING A PHASE IB CLINICAL TRIAL IN BENINESE NULLIGRAVID WOMEN

Tatiana Sandrine Hountohotegbe[1], Darius Sossou[2], Jean Claude Fan[3], Jeremie Koffi[3], Komi Gbedande[4], Firmine Viwami[5], Florentin Aussenac[6], Saadou Issifou[7], Euripide Avokpaho[6], Morten Nielsen[8], Benjamin Mordmüller[9], Odile Leroy[10], Achille Massougbodji[2], Nadine Fievet[6], Adrian Luty[6]

[1]*Université de Paris, MERIT, IRD, F-75006. Institut de Recherche Clinique du Bénin. Centre d'Etude et de Recherche sur le Paludisme Associé à la Grossesse et à l'Enfance, Paris, France,* [2]*Centre d'Etude et de Recherche sur le Paludisme Associé à la Grossesse et à l'Enfance, Cotonou, Benin,* [3]*Département de Biosciences, Unité de Formation et de Recherche en Pharmacie, Université Félix Houphouët Boigny, Abidjan, Côte D'Ivoire,* [4]*University of Texas Medical Branch, galveston, TX, United States,* [5]*Centre d'Etude et de Recherche sur le Paludisme Associé à la Grossesse et à l'Enfance, cotonou, Benin,* [6]*Université de Paris, MERIT, IRD, F-75006, Paris, France,* [7]*Institut de Recherche Clinique du Bénin, cotonou, Benin,* [8]*Department of Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark,* [9]*Institut für Tropenmedizin, Eberhard Karls Universität Tübingen, Tübingen, Germany,* [10]*European Vaccine Initiative, Heidelberg, Germany*

Placental malaria is characterised by the sequestration of parasite-infected erythrocytes in the maternal intervillous blood spaces of the placenta, leading to low birth weight, abortion, maternal anaemia during pregnancy, and increased susceptibility to *P. falciparum* in infancy. Placental sequestration is mediated by VAR2CSA, a parasite antigen that interacts with chondroitin sulfate A on syncytiotrohoblasts. One vaccine strategy is to block this interaction through generation of VAR2CSA-specific antibodies. PAMVAC is a VAR2CSA protein-based vaccine candidate, comprising the minimal CSA binding domain located in the N-terminal ID1-DBL2-ID2 domain. The preliminary results of the Phase I trial revealed the presence of antibodies that inhibit the binding of VAR2CSA-expressing *P. falciparum*-infected erythrocytes to CSA in unexposed healthy adult volunteers. It is well-established that long-lived, high-affinity antibody responses induced by vaccines depend on the generation of long-lived memory T cells. However, the mechanisms through which such memory cells support this antibody-mediated immunity against placental malaria is poorly understood. Here, T cell phenotype and function were assessed by FACS using peripheral blood mononuclear cells (PBMC) collected from 21 healthy nulligravid adult Beninese during the Phase Ib PAMVAC vaccine trial. PBMC of primigravid Beninese followed longitudinally in a separate study of malaria during pregnancy were used for comparison. The T cell subsets and specific cytokine production profiles were compared by time-point, adjuvant, and group. These data provide further insights into VAR2CSA-specific T cell responses to placental malaria acquired both during natural exposure and via vaccination and should contribute to improving current vaccine strategies.

803

WORLDWIDE GENETIC ANALYSES OF MAJOR MALARIA PREERYTHROCYTIC STAGE ANTIGENS IDENTIFIED VACCINE CANDIDATE VARIANTS AND EPITOPES

Amed Ouattara[1], Ankit Dwivedi Ankit[2], Matthew Adams[1], Matthew B. Laurens[1], Matthew B. Laurens[1], Amadou Niangaly[3], Myaing M. Nyunt[4], Christopher V. Plowe[4], Abdoulaye Djimde[3], Shannon Takala-Harrison[1], Joana C. Silva[2]

[1]*Center for Vaccine Development and Global Heath, University of Maryland Baltimore, baltimore, MD, United States,* [2]*Institute for Genome Sciences, University of Maryland School of Medicine, baltimore, MD, United States,* [3]*Malaria Research and Training Center, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali,* [4]*Duke Global Health Institute, Duke University, Durham, NC, United States*

Failure to account for genetic diversity of antigens during vaccine design may lead to vaccine escape, wherein efficacy is directed against immunogenically similar strains and/or variants, limiting overall efficacy. To evaluate vaccine escape potential of antigens currently in development or clinical testing, we surveyed the genetic diversity of ten *Plasmodium falciparum* preerythrocytic stage antigens using whole genome sequence data from 1,010 field isolates obtained from the public domain (721) or generated in-house (289). Of these, 699 were collected in Africa (Burkina Faso, Cameroon, Guinea, Kenya, Malawi, Mali, and Tanzania), 69 in South America (Brazil, Colombia, French Guiana, and Peru), 59 in Oceania (Papua New Guinea), and 183 in Asia (Cambodia, Myanmar, and Thailand). Antigens surveyed include cell-traversal protein for ookinetes and sporozoites (CelTOS), circumsporozoite protein (CSP), liver stage antigens 1 and 3 (LSA1, LSA3), sporozoite surface proteins P36 and P52, sporozoite asparagine-rich protein-1 (SAP1), sporozoite microneme protein essential for cell traversal-2 (SPECT2), thrombospondin-related anonymous protein (TRAP), and upregulated in infectious sporozoite 3 and 4 (UIS3 and UIS4). For each antigen, we estimated genetic diversity, performed neutrality tests to detect positive selection, F_{ST} analyses to measure population differentiation, and *in silico* prediction and analysis of B- and T-cell epitopes. We found that UIS3, UIS4 and P36 variants were not constricted by regions/continents, including a few that when combined would be representative of the worldwide parasite population. Moreover, we identified predicted B- and T-cell epitopes conserved in CELTOS, CSP, LSA1, LSA3, and SPECT2, and could be further explored in immunogenicity and for protective efficacy. These epitopes may ultimately be considered for the design of a liver stage multi-peptide malaria vaccine. These findings provide crucial evidence for the rational design of a liver stage multivalent malaria vaccine that covers the global genetic polymorphism observed in *P. falciparum*-endemic areas.

804

A COBALT PORPHYRIN LIPOSOMAL ADJUVANT WITH REDUCED MPLA, CAPABILITY FOR LYOPHILIZATION AND RE-CONSTITUTION, AND THERMOSTABILITY

Moustafa Mabrouk[1], Wei-Chiao Huang[1], Bingbing Deng[2], Nasi Li-Purcell[3], Gunes Ekin Atilla-Gokcumen[3], Amal Seffouh[4], Carole Long[2], Joaquin Ortega[4], Kazutoyo Miura[2], Jonathan Lovell[1]

[1]*Department of Biomedical Engineering, University at Buffalo, State University of New York, Buffalo, NY, United States,* [2]*Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States,* [3]*Department of Chemistry, University at Buffalo, State University of New York, Buffalo, NY, United States,* [4]*Department of Anatomy and Cell Biology, McGill University, Montreal, QC, Canada*

Improved thermostability and decreased vaccine component cost would facilitate large-scale rollouts of future vaccines intended for sub-Saharan Africa, where most worldwide deaths from HIV and malaria occur. We previously showed that a model malaria vaccine candidate antigen, Pfs25, can be rendered more immunogenic, with dose-sparing potential, when mixed with liposomes containing cobalt porphyrin-phospholipid "CoPoP" and a synthetic monophosphoryl lipid A (MPLA) variant, as these liposomes induce stable particle formation of the antigen. In the present work, different synthetic MPLA variants were assessed in liposomal formulations and liposome physical stability and immunogenicity were not adversely impacted with a 60 % reduction in MPLA content. When admixed with Pfs25, these adjuvant formulations induced functional antibodies in mice and rabbits. Lyophilized antigen-liposome formulations were developed using sucrose or trehalose cryoprotectants, which improved vaccine reconstitution for a variety of model antigens that were pre-bound to the liposomes. Lyophilized liposomes exhibited improved chemical stability and retained capacity for binding antigens. Compared to liquid storage, lyophilized Pfs25 CoPoP liposomes exhibited thermostability with respect

to size, binding capacity, protein folding and immunogenicity. Following 6 weeks of storage at 60 °C (the longest storage period assessed) and mouse immunization, the lyophilized formulation induced functional antibodies in mice.

805

A PHASE 1B, OPEN-LABEL, AGE DE ESCALATION, DOSE ESCALATION STUDY TO EVALUATE THE SAFETY AND TOLERABILITY OF DIFFERENT DOSES OF A CANDIDATE MALARIA VACCINE ADJUVANTED R21(R21/MM) IN ADULTS, YOUNG CHILDREN AND INFANTS IN KILIFI, KENYA

Irene W. Njau[1], Mehreen Datoo[2], Samuel Sang[1], Caroline Ngetsa[1], Katie J. Ewer[2], Francis Ndungu[1], Melissa Kapulu[1], Philip Bejon[1], Adrian V. Hill[2], Mainga Hamaluba[1]

[1]*KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya, Kilifi, Kenya,* [2]*Jenner Institute, Oxford University, UK, Oxford, United Kingdom*

There is an urgent need for an efficacious malaria vaccine. Whilst RTS, S/AS01 is the only malaria vaccine in advance clinical development, efficacy is suboptimal in infancy and some safety concerns remain under evaluation. R21 adjuvanted to Matrix-M (R21/MM) is a promising candidate malaria vaccine as assessed in pre-clinical and phase 1a trials (UK, Burkina Faso). We conducted a Phase Ib open-label, age de-escalation, dose-escalation study to evaluate the safety and immunogenicity of different doses of R21/MM in adults, young children and infants in Kilifi, Kenya. We recruited 20 adults (18-45 years), 20 children (1-5 years) and 51 infants (5-<12 months) and administered either a full (10mcg R21/50mcg MM), intermediate (5mcg R21 in 50mcg MM) or half (5mcg R21/25mcg MM) dose of the vaccine. All groups received three doses of the vaccine four weeks apart and had safety bloods at screening and on days 2 and 7 post vaccination. Participants were visited at home by a fieldworker in the week following each vaccination to elicit adverse events. Solicited and unsolicited adverse events were assessed in the week following vaccination and at each vaccination visit. Serious adverse events collated throughout the study. Median age of adults was 27, children and infants 34.5 and 8 months respectively. The vaccine was very well tolerated across age groups. Solicited adverse events were mild and short-lived across all groups and doses. There were no clinically significant blood results related to vaccination in the week following vaccination. Amongst adults, the most frequently reported local solicited adverse event was pain at the injection site (75%) whereas pain was only reported in 5% of the target population - infants. The most frequent adverse event in infants was fever (25.5%, resolving before 48hrs). No fevers were reported in the adults following vaccination. 2 serious adverse events have been reported, both respiratory illnesses in children, unrelated to vaccination and have since resolved. Our results suggest that R21/MM is safe and well tolerated and warrants further safety and efficacy evaluation in phase II and III trials.

806

ATTRIBUTING SPATIAL REPELLENT EFFICACY TO ENTOMOLOGICAL EFFECTS MODULATED BY HUMAN BEHAVIOR

Annaliese Wieler, Nicole L. Achee, John P. Grieco, Neil F. Lobo, Sean Moore, Alex Perkins

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

Though malaria incidence has been declining worldwide over the past century due to various control measures, progress has slowed in the past few years. Among other challenges, this slowdown has been attributed to insecticide and drug resistance and gaps in human protection from potentially infectious mosquitoes. Continuation of the downward trajectory of malaria incidence will require new or more optimized products, and/or delivery strategies that can address gaps in protection against transmission. Spatial repellents, an intervention class comprising products that emanate volatile chemicals, have been evaluated in large-scale clinical trials for efficacy against malaria. These product types have been shown in laboratory and semi-field studies to reduce mosquito-human contact by interfering with home entry, host seeking, and/or blood feeding behaviors. Although entomological effects have been integrated into clinical trials, these objectives are secondary. Here we present Bayesian inferences using a Ross-MacDonald transmission model to assess how contribution of decreased mosquito lifespan and decreased human biting effects decreases in malaria incidence that have been observed in a clinical trial. These inferences were informed by four data sources that informed the model's likelihood: malaria incidence, human landing catches, mosquito parity (estimate of previous blood meal), and sporozoite positivity (infectiousness). We placed empirically informed priors on the model's parameters using historical data and sampled from the posteriors of model parameters to determine what proportion of malaria reduction was attributable to each entomological effect. We also used data on time spent indoors or outdoors by trial participants to explore how human behavior during mosquito biting hours modulated the efficacy of a spatial repellent product. Inferences are intended to enhance model projections on the impact of spatial repellent products if the intervention class is recommended for deployment as a malaria control strategy at broader, ecologically diverse scales.

807

ENTOMOLOGICAL AND HUMAN BEHAVIORAL ASSESSMENT IN FIVE HIGH MALARIA ENDEMIC REGENCIES IN PAPUA PROVINCE, INDONESIA

Dendi Hadi Permana[1], Lepa Syahrani[1], Ismail Ekoprayitno Rozi[1], Puji Budi Asih[1], Siti Zubaidah[1], Rifqi Risandi[1], Suradi Wangsamuda[1], Nancy Dian Angraeni[2], Marthen Robaha[3], Pargomgoman Panjaitan[4], Pungut Sunarto[5], Johann Albert Tokoro[6], Taufiq Imansyah Syaifat[7], R. Samson Manao[8], Maria Endang Sumiwi[9], Michael J. Bang[10], Neil F. Lobo[11], William A. Hawley[9], Din Syafruddin[1]

[1]*Eijkman Institute for Molecular Biology, Central Jakarta, Indonesia,* [2]*Sub directorate of Malaria, Ministry of Health, Central Jakarta, Indonesia,* [3]*Malaria Section, Papua Provincial Health Department, Papua, Indonesia,* [4]*Malaria Section, Keerom Regency Health Department, Papua, Indonesia,* [5]*Malaria Section, Jayapura Regency Health Department, Jayapura, Indonesia,* [6]*Malaria Section, Sarmi Regency Health Department, Papua, Indonesia,* [7]*Malaria Section, Boven Digoel Regency Health Department, Papua, Indonesia,* [8]*Malaria Section, Mimika Regency Health Department, Papua, Indonesia,* [9]*United Nations Children's Fund, Central Jakarta, Indonesia,* [10]*PT Freeport Indonesia, International SOS, Freeport Medical Services., Jakarta, Indonesia,* [11]*Eck Institute for Global Health, University of Notre Dame, Notre Dame, IN, United States*

Malaria cases remains high in eastern Indonesia. To expedite malaria elimination efforts, rapid entomological assessments and human behavioural observations were conducted in five regencies in Papua, Indonesia. The objective is to characterize focal and specific entomological findings for understanding the transmission dynamics, where interventions may function best, and identify gaps in individual and community-based protection. Assessments included identification of vector using human landing collections (HLC), resting collections, identification of larval habitats, vector incrimination, and human behavior related to transmission risk. The HLC revealed the primary vector species as members of the *Anopheles punctulatus* group (*An. farauti*, *An. hinesorum*, *An. koliensis*, *An. punctulatus*). All displayed indoor and outdoor biting behavior. Other less frequent species (e.g., *An. longirostris*) was possibly secondary vectors. Only two species were found with sporozoites, *An. koliensis* and *An. punctulatus* collected in Keerom, Jayapura and Sarmi regencies. Larval collections identified both permanent and temporary natural water collections within and surrounding sampled villages. Human behavior observations identified gaps in protection, both indoor and outdoor. All locations had multiple potential vector species and larval habitats in close proximity to human habitation. The HLC findings confirm previous observations on behaviour of these species and reaffirm the high likelihood of malaria transmission outdoors. This explains, in part, why current malaria control efforts fail to reduce disease incidence in the regencies. Guided community participation in larval habitat reduction

practices is one recommended remedy to reduce overall transmission risk. In addition, adoption of personal mosquito protection measures during outdoor activities should be promoted.

809

IMPLEMENTATION OF A CONTINGENCY PLAN AFTER WITHDRAWAL OF INDOOR RESIDUAL SPRAYING (IRS) IN THE COMMUNES OF KEROU AND PEHUNCO OF ATACORA DEPARTMENT IN NORTHERN BENIN, WEST AFRICA

Jean Fortune Dagnon[1], B. Onambele[2], P. Condo[1], J. Norin[3], F. Challa[1], F. Niyuhire[4], F. Niyuhire[4], L. Zinsalo[3], M. Postma[5], R. Aikpon[6], A. Salako[7], I. B. Ahogni[7], B. Akinro[7], S. Chitou[7], M. Akogbeto[7], A. Ogouyemi-Hounto[7], R. Van Huslt[8]

[1]*US President's Malaria Initiative, US Agency for International Development, Cotonou, Benin,* [2]*US Agency for International Development, Cotonou, Benin,* [3]*USAID ISHA Project, Porto Novo, Benin,* [4]*USAID ISHA Project, Cotonou, Benin,* [5]*Department of Health Sciences, University of Groningen, University Medical Center Groningen (UMCG), Groningen, Netherlands,* [6]*National Malaria Control Program, Ministry of Health, Cotonou, Benin,* [7]*Centre de Recherche Entomologique de Cotonou (CREC), Cotonou, Benin,* [8]*Department of Economics, Econometrics & Finance, University of Groningen, Faculty of Economics & Business, Groningen, Netherlands*

In 2019, after seven annual campaigns of indoor residual spraying (IRS) in Atacora department, the final phase of IRS withdrawal from Atacora occurred in the two remaining communes of Pehunco and Kerou. An IRS Contingency Plan (ICP) was implemented to identify and mitigate the resurgence of malaria cases due IRS withdrawal. The ICP implementation in Pehunco and Kerou covered 78 villages, 26 health facilities and 2 hospitals from May to December 2019 using 4 essential strategies: 1) Weekly epidemiological sentinel surveillance, 2) re-enforced case management, 3) social and behavior change/community-based activities, and 4) supply chain audits and commodity stock up. The Wilcoxon non-parametric test was used to determine statistical difference between malaria cases pre-IRS withdrawal and post-IRS withdrawal from 2017 to 2019. Weekly sentinel surveillance initiated in 10 health facilities, showed there was a significant increase of uncomplicated malaria cases in 2019 of 63.7% and 168.4% compared to cases observed in 2018 and 2017 respectively, for all ages and children under five of age (p=0.001). For severe malaria cases, a 470.6% and 603.5% increase in cases was observed in 2019 compared to 2018 and 2017 respectively, for both ages' groups (p=0.37). To mitigate the upsurge in malaria cases, 15 health providers were retrained on the diagnosis and treatment of severe and uncomplicated malaria, and 95 community health workers on the delivery of prevention messages. Two sessions of Outreach Malaria Test and Treat Strategy (OMTTS) were conducted; a total of 2283 malaria cases were identified and treated in high resurgence villages. An IRS withdrawal communication plan was developed and implemented that reached more than 10,000 people. Lastly, regional medical warehouses were supported to ensure constant availability of malaria commodities. Logistic limitations and timing prevented mass distribution of insecticide treated nets, however, continuous distribution was in place. Despite implementing ICP, there was still an upsurge of malaria cases. Further analyses would be useful to quantify the extent to which ICP limited malaria cases.

810

RESIDUAL EFFICACY OF ACTELLIC 300CS (PIRIMIPHOS-METHYL) AND SUMISHIELD 50WG (CLOTHIANIDIN) USED FOR IRS IN MALI IN 2018-2019

Libasse Gadiaga[1], Chitan Keita[1], Sinaba Youssouph[1], Therese Dembele[1], Abdourahamane Dicko[2], Jules Mihigo[3], Aliou Diallo[3], Jenny Carlson[4], Cecilia Flatley[5], Richard Oxborough[5]

[1]*VectorLink, Bamako, Mali,* [2]*NMCP, Bamako, Mali,* [3]*PMI, Bamako, Mali,* [4]*PMI, Washington, WA, United States,* [5]*Abt Associate, Rockville, MD, United States*

Since 2008, PMI has supported targeted IRS for malaria control in high-burden areas of Mali. From 2017, PMI IRS operations have been conducted in Mopti Region in central Mali, where a 2015 study showed 60 percent malaria prevalence compared to 30 percent nationally. In 2018 and 2019 two insecticides formulations Actellic 300CS (pirimiphos-methyl) and SumiShield 50WG (clothianidin) -- were used for IRS. Monthly cone bioassays were conducted on sprayed walls following WHO protocols using an insectary colony of pyrethroid-susceptible *An. coluzzii*. In each district, 5-10 randomly selected houses were tested with a total of 30 mosquitoes per house. Mortality rate was recorded 24 hours after exposure to Actellic 300Cs and 24, 48 and 72 hours after exposure to SumiShield 50WG to capture any delayed mortality. Data were interpreted using WHO guidelines, considering insecticide effective with mortality &gt 80%. In 2018, Actellic 300CS was effective for 3 months on mud and cement walls in Bandiagara District, but 4 and 5 months on mud and cement walls, respectively, in Bankass District. In 2019, Actellic 300CS remained effective for 4 months on mud and 7 months on painted cement in Mopti District, but 6 and 7 months, respectively, on cement and mud in Bandiagara District. In 2018, SumiShield WG provided at least 9 months (when testing stopped) residual efficacy on all wall types after a 24-hour holding period in Mopti District, 4 months using 24-hour mortality and 9 months using 48-hour mortality in Djenné District. In 2019, SumiShield had 4-5 months residual efficacy using 24-hour mortality, 5-7 months using 48-hour mortality, and 7 months using 72-hour mortality on all wall types. Overall, Actellic CS provided between 3 and 7 months of residual duration within 24 hours post-exposure and SumiShield WG provided between 4- and 9-months residual efficacy considering 24-hour mortality and at least 7 to 9 months if considering 72-hour mortality. SumiShield WG should be the primary formulation used for IRS in Mali due to longer residual duration, but Actellic CS should be used as part of a resistance management strategy as well as other products such as Fludora Fusion WP-SB.

811

GOOGLE EARTH ENGINE AND QUANTILE REGRESSION FORESTS; A COST-FREE GENERIC FRAMEWORK TARGETING HOTSPOTS OF MALARIA MOSQUITO ABUNDANCE AND A TRAINING MANUAL FOR NON-SPECIALISTS

Clare Strode[1], Clare Rowland[2], Aneurin W. O'Neil[2], Seth R. Irish[3], Francis Wat'senga[4], Paul Aplin[1], Chris G. Marston[2]

[1]*Edge Hill University, Ormskirk, United Kingdom,* [2]*UK Centre for Ecology and Hydrology, Lancaster, United Kingdom,* [3]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [4]*National Institute of Biomedical Research, Kinshasa, Democratic Republic of the Congo*

Anopheles mosquitoes are vectors of malaria, a disease responsible for a significant burden of global disease and nearly half a million deaths in 2018. In this study, methods using Quantile Regression Forests (QRF), cost-free Earth Observation (EO) data, Google Earth Engine (GEE), and *in-situ* longitudinal mosquito monitoring and land cover data were developed to identify the bio-geographical variables driving the abundance and distribution of malaria vectors. The study investigated population abundance of *Anopheles gambiae s.l., An. funestus* and the secondary malaria vector *An. paludis* in highly malaria endemic areas (Lodja and Kapalowe) in the Democratic Republic of the Congo (DRC). Here, optical imagery from the Sentinel-2 satellite, which is restricted by cloud cover,

is used synergistically with radar data from the Sentinel-1 satellite which can penetrate cloud, overcoming historical constraints in persistently cloudy, tropical and sub-tropical environments. We found that substantial spatio-temporal variability occurred in predicted mosquito abundance in the two regions, and that the climatic and land cover variables influencing mosquito distributions varied between mosquito species and site. Rainfall was consistently of high importance in relation to abundance. Predictive application of QRF models generated monthly abundance maps for each species, identifying both spatial and temporal 'hot-spots' of high abundance, and by proxy, increased malaria infection risk. These maps will provide a crucial tool for improving targeting of resource-constrained disease control activities to reduce malaria burdens in endemic regions. The methods developed are designed to be widely applicable to other areas, where suitable *in-situ* mosquito monitoring data are available. From this study we have produced a user manual and training materials enabling independent implementation of these methods. The manual and training material will be openly available, enabling local agencies to implement these methods which removes the reliance on external EO specialists and high-performance IT infrastructure unavailable in many DAC countries.

812

A COMBINATION OF METABOLIC RESISTANCE AND 1014F MUTATION IS INVOLVED IN THE PYRETHROID RESISTANCE OF *ANOPHELES GAMBIAE* IN SENEGAL

Oumou K. Gueye[1], Abdoulaye K. Dia[1], Mouhamed B. Faye[1], Ousmane Faye[1], El Hadj A. Niang[1], Charles S. Wondji[2]

[1]*Universite Cheikh Anta Diop, Dakar, Senegal,* [2]*Vector Biology Departement, Liverpool School of Tropical Medicine, Pembroke place, United Kingdom*

The emergence and spread of Pyrethroid resistance in the main Malaria vector *Anopheles gambiae s.s.* is a real threat to the effectiveness of the malaria vector control in Senegal. Previously Kdr mutation was initially found only in *An. gambiae*, but later on in its counterpart *An. coluzzii* across their sympatric distribution range. Here we investigated the putative involvement of the target site Kdr mutation as well as of metabolic mechanisms in the phenotypic resistance of both species. *An. gambiae s.l.* mosquitoes were collected in Fatick (an allopatric or predominance area of *An. coluzzii*), Kedougou (an allopatric or predominance area of An. gambiae) and Tambacounda (a sympatric area for both species) during the raining season of 2017 and 2018. WHO resistance bioassays were conducted on 3-5 days old unfed females. Synergist assays with the piperonyl-butoxide (PBO) were additionally performed in 2018. Molecular identification of the *An. gambiae* s.l. species and the Kdr mutations were genotyped by PCR. The expression of putative genes involved in metabolic resistance was also assessed by the qPCR.Tested populations were resistant to pyrethroid and DDT (mortality ≤ 90%) but susceptible to organophosphate and carbamate (mortality ≥ 98%) in all the study sites, except in Kedougou where suspected resistance to Bendiocarb (mortality: 93.3±3%) was noted. Pre-exposure to PBO, induced a partial recovery of tested population susceptibility to permethrin and a total recovery to deltamethrin. The 1014F mutation was found in both species with the highest frequency in *An. gambiae*. The overexpression of CYP6Z1 and CYP6Z2 in resistant study populations compared to the susceptible strain suggest their involvement in the resistance *An. gambiae* to pyrethroid. Our study revealed the widespread presence of 1014F mutation in both sympatric *An. gambiae* and *An. coluzzii* population and the implication of gene expression on An. gambiae pyrethroid resistance. The PBO-LLINs and the use of organophosphate and carbamates molecules for IRS may be an alternative solution to control pyrethroid resistant populations in this malaria endemic study areas.

813

HOUSE SUITABILITY FOR THE IMPLEMENTATION OF EAVE TUBES FOR MALARIA VECTOR CONTROL IN IVORY COAST

Tiago Canelas[1], Edward Thomsen[1], Daniel McDermott[1], Eleanore Sternberg[1], Matthew E. Thomas[2], Eve Worrall[1]

[1]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [2]*Department of Entomology and Center for Infectious Disease Dynamics, The Pennsylvania State University, University Park, PA, United States*

Eave tubes (ET) is a promising vector control tool to target malaria and possibly other vector-borne diseases. ET are a section of pipe fitted into a closed eave, with an electrostatically coated netting that binds powder formulations of insecticide inside the pipe. In combination with closing eaves and sealing cracks, ET reduces mosquito entrance while maintaining airflow. However, houses must be made with materials that allow the ET installation. A cluster randomized controlled trial of screening plus eaves tubes has been conducted in several villages in Ivory Coast with promising results. Additional evidence is required to support scale-up decisions in different settings, with one question being around housing suitability for ET. We applied a geostatistical model to estimate house suitability throughout Ivory Coast. We categorized the DHS house materials on suitability and calculated the percentage of suitable households within a DHS cluster. To achieve community efficacy, trial data suggests a minimum of 70% of the houses in the clusters need ET, hence we assumed an 80% suitability threshold to allow for refusals. We calculated the exceedance probabilities of house suitability at 5 by 5 km spatial scale and input the geostatistical results into a fuzzy logic model to determine the area's best suited to ET. Overall, 45% of the clusters met the threshold of being comprised of at least 80% suitable households. However, there was high spatial heterogeneity throughout the country and only 5.8% of the suitable clusters are in rural areas. Ivory Coast contains few suitable areas that overlap with high or medium malaria transmission intensity. These areas are concentrated in a south to north axis around the big cities. Other studies have estimated an improvement of house materials across Africa in recent years. Therefore, this model could underestimate the true distribution of suitable areas. Nevertheless, this information provides the first attempt to determine the suitability of ET on a national scale and a pilot study to model on a continental scale. Results are at the subdistrict level to facilitate use in decision-making by the control programme.

814

IMPLICATIONS OF ENTOMOLOGICAL SURVEILLANCE DATA FOR SELECTION OF OPTIMAL VECTOR CONTROL INTERVENTIONS IN NAMIBIA

Itula Itula[1], Deodatus Maliti[1], Stark T. Katokele[1], Ophilia Lukubwe[1], Mwema Tabeth[2], Rosalia Joseph[2], Dennis Walusimbi[3], Erica Paige Berlin[3], Sheila Barasa Ogoma[3], Elodie Vadja[4], Neil Francis Lobo[5], Joseph Zvoushoma[3], Ayokunle Abogan[3], Allison Tatarsky[4], Cara Smith Gueye[4], Petrina Uusiku[1]

[1]*Namibia National Vector-Borne Disease Control Programme, Ministry of Health and Social Services, Windhoek, Namibia,* [2]*University of Namibia, Windhoek, Namibia,* [3]*Clinton Health Access Initiative, Boston, MA, United States,* [4]*UCSF Malaria Elimination Initiative, San Francisco, CA, United States,* [5]*University of Notre Dame, South Bend, IN, United States*

Namibia has re-oriented its malaria policy from control to elimination through with a goal of eliminating malaria by 2022. In the past fifteen years, a significant reduction in malaria burden has been recorded with incidence dropping from 300 per 1000 population in 2004, to 1.3 in 2019. Namibia relies on Indoor Residual Spraying (IRS) as the main intervention with Long Lasting Insecticidal Nets (LLINs) and Larval Source Management (LSM) implemented supplementary to IRS. From 2018, Namibia introduced annual entomological surveillances in all 9 endemic regions with the aim of identifying entomological drivers of transmission and gaps in existing vector control interventions. Indoor and outdoor malaria vector species composition and human biting rate were measured using Human Landing Catches (HLC) in selected households across 12 hours of the

night between March and May 2018 and 2019. In addition, indoor vector resting density was studied using Pyrethrum Spray Catches (PSC). Standard WHO test procedures were used to study insecticide resistance status of malaria vectors from endemic regions. Results show co-existence of the two major malaria vectors *Anopheles arabiensis* (56.8%) and *An. gambiae* sensu stricto (41.0%), with *An. funestus* s.s. (2.3%) as secondary vector; all vectors showing high propensity towards outdoor biting (70-90%). Indoor resting density of the major vectors was relatively low (0.6 mosquitoes/house/night). Widespread development of insecticide resistance was confirmed in almost all endemic regions especially against Deltamethrin and to a lesser extent against DDT. The re-appearance of *An. gambiae* s.s. is significant due to its specialized role in driving malaria transmission indoors. Malaria elimination goal is challenged due to lack of effective tools for preventing outdoor malaria transmission. These results will inform the choice of insecticides to be used in a rotation plan as mitigation against emerging insecticide resistance. Results from this study will also be used to inform Namibia's Integrated Vector Management (IVM) strategy, including decisions about supplementing IRS with LLINs and LSM.

815

BBNETS - REFINING BARRIER BEDNETS FOR EFFECTIVE MALARIA VECTOR CONTROL IN THE POST-PYRETHROID ERA

Philip J. McCall

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Widespread use of insecticidal bednets (ITNs) was the primary method used to achieve the remarkable reductions in malaria transmission recorded between 2000 and 2015. Today, those standard pyrethroid-treated bednets have lost efficacy against the pyrethroid-resistant vector populations that are now widespread throughout Africa. Ensuring a future for ITNs requires insecticides that do not share resistance mechanisms with pyrethroids and also are suitable for safe delivery via ITNs. One potentially simple solution is the barrier bednet (BBnet). Essentially, this is a standard rectangular bednet with an additional insecticidal panel or barrier projecting into the space above the bednet roof where *Anopheles gambiae* activity is high. Mounted on the roof, barrier panels have zero contact with sleepers, thus permitting application of higher dosages or a broader range of insecticides than currently possible. Tested against wild pyrethroid-resistant *An. gambiae* in Burkina Faso, standard pyrethroid bednets with organophosphate barriers achieved significantly higher killing rates than bednets alone. Further studies have tested additional barrier treatments and evaluated the performance of different barrier shapes and sizes mounted on standard bednet bases. We have used infra-red video tracking to explore how the barrier targets and kills mosquitoes, and whether apparent changes in flight behaviour of *An.gambiae* induced by the presence of the barrier are involved. We also tackled the challenge of maintaining an erect barrier over the lifespan of the net, essential for optimising the BBnet's performance. Using existing insecticide formulations, a prototype BBnet will be ready for evaluation in hut trials and small-scale community trials in late 2020, with the hope of accelerating its progress towards WHO approval and early deployment in at-risk communities throughout Africa

816

KEY CHALLENGES AND LESSONS LEARNED FROM THE IMPLEMENTATION OF BEDNETS MASS DISTRIBUTION IN THE REPUBLIC OF CONGO, 7 YEARS AFTER THE LAST CAMPAIGN

Njara Rakotonirina[1], Jean-FLorent Mbanza[1], Jean-Mermoz Youndouka[2]

[1]*Catholic Relief Services, Brazzaville, Republic of the Congo,* [2]*National Malaria Control Program, Brazzaville, Republic of the Congo*

The Republic of Congo conducted a mass distribution of Long-Lasting Insecticide-treated Nets (LLINs) in 2012, and the renewal was not implemented until 2019. Between 2015 and 2018, malaria hospitalization increased from 4.05 to 7.87 per 10,000, and malaria mortality from 0.06 to 0.16 per 100,000. In 2014, the ownership and usage rates of LLINs were respectively at 27% and 60%. Funded by the Global Fund (GFATM), the 2019 campaign was executed under the leadership of the National Malaria Program, with the Catholic Relief Services as the principal recipient. LLINs were ordered as soon as the grant was signed in May 2018. Trainings of over 6000 community actors have followed, to prepare the micro-planning workshops and the household registration, for 52 health districts to cover 5 million population. Challenges were encountered in getting reliable data per district, making difficult the micro-planning and the needs estimation for the registration, logistics, and communication activities. Transportation was one of the key challenges, considering the rainy season and difficult access to some districts. The communication plan was a challenging aspect resulting from the limited resource invested, qualitative surveys have shown correlation between lack of awareness and rejection of the registration. Social media rumors against LLINs impacted the campaign, and specific communication, including social media responses and civil society interventions, were implemented to minimize rejections. Despite the challenges, 2,602,556 LLINs have been distributed by December 2019, reaching 85% of national coverage. Out of 12 regions, 9 reached a coverage higher than the average, including Bouenza at 101%, Lekoumou and Cuvette-Ouest at 97%. However, 3 regions were lower than the average: 81% in Pointe-Noire, 75% in Sangha and 26% in Likouala. Given the size of Brazzaville and Pointe Noire, representing 70% of the country's population, a catch-up distribution was organized in 2020 in these 2 regions, along with the Likouala and Sangha. This analysis aims to explore the most important challenges and lessons that impacted the campaign, and the solutions adopted.

817

MALARIA VECTOR BIONOMICS & RESISTANCE PATTERNS IN 2018 AND 2019 IN NIGER

Soumaila Hadiza[1], Hamani Boube[2], Daouda Abdoulaye[2], Iro Souleymane[2], Samira Abdoulaye Gouro[2], Mahamane Sani Amadou Zaman Allah[2], Fatimata Abdoulaye Daouda[2], Ibrahim Issa Arzika[3], Soumana Amadou[3], Izamne Mahamadou[3], Saadou Kadri[3], Mamane Sale Noura[3], Hounkanrin Zoulkifouly Wilfried[3], Rabiou Labbo[3], Hadiza Jackou[2], Sabiti Idrissa[2], Els Mathieu[4], Ellen Dotson[5], Cecilia Flately[6], Dereje Dengela[6], Joseph Chabi[7]

[1]*VectorLink Niger Project, Niamey, Niger,* [2]*National Malaria Control Programme, Niamey, Niger,* [3]*Centre de Recherche Médicale et Sanitaire, Niamey, Niger,* [4]*U.S. President's Malaria Initiative, Niamey, Niger,* [5]*U.S. Centers for Disease Control and Prevention, Atlanta, GA, USA, Niger PMI VectorLink Project, Atlanta, GA, United States,* [6]*PMI VectorLink Project, Rockville, MD, United States,* [7]*PMI VectorLink Project, Abdjan, Côte D'Ivoire*

Very limited entomological data are available to inform vector control decisions in Niger. From August 2018 to March 2019, nationwide comprehensive entomological monitoring was conducted in thirteen sites. Adult mosquitoes were collected using human landing catches and pyrethrum spray catches to determine vector species composition, human biting rates, indoor resting densities, parity, & entomological inoculation rate. Susceptibility tests against alpha-cypermethrin, deltamethrin, permethrin, bendiocarb, pirimiphos methyl, resistance intensity tests & synergist assays were conducted with *Anopheles gambiae* s.l. using WHO susceptibility test kits & CDC bottle assays. In all sites except Guidimouni, *An. gambiae* complex was the predominant vector ranging from 62 to 100% of the *Anopheles* species collected & *An. coluzzii* was the most abundant member (83-100%) of the complex. *An. funestus* s.l. represented 80% of species collected in Guidimouni and 13% in Zindarou. The average biting of *An. gambiae* s.l. ranged from 12.5 to 109.8 bites/person/night. The mean indoor resting density was 17 *An. gambiae* s.l. per house and mean parity ranged between 51.5% & 81.7%. The average annual EIR was 92.9 infected bites per person nationwide, ranging from 0 to 367.3. In 2019, insecticide susceptibility to all three pyrethroids in all sites ranged from 0% to 60%. Mosquitoes were resistant to 5x concentrations of pyrethroids at all sites & full susceptiblity to 10x permethrin, and alpha-cypermethrin was observed at only two sites (Gaya & Tessaoua). PBO restored susceptibility to pyrethroids to varying

degrees: for deltamethrin, increases in mortality ranged from 30 to 70%; for permethrin, 0 to 60%; and for alpha-cypermethrin, 5 to 70%. Bendiocarb susceptibility ranged from 78 to 99% and susceptibility to pirimiphos-methyl was above 98% in all but one site. Mortality 72 hours after exposure to 200µg/bottle of chlorfenapyr was 100% at all sites. Data gathered over time in Niger will support the development of a critical knowledge base to optimize the appropriate and timely allocation of vector control resources in Niger.

818

IMPROVING UPTAKE OF LONG-LASTING INSECTICIDE TREATED NETS AMONGST ELIGIBLE PREGNANT WOMEN AND CHILDREN IN RESOURCE LIMITED SETTINGS-LESSONS LEARNED FROM IMPLEMENTING A MIX TARGETED INTERVENTION

Frank Oronsaye[1], Chidinma Okolo[1], Temitope Adeyoju[1], Chineddu Chukwu[2], Jane Onyilo[3], Olatayo Abikoye[1], Ezinne Onuba[1], Peter Biaghere[1], Perpetua Uhomoibhi[4], Ibrahim Maikore[4], Diwe Ekweremadu[1], Sonachi Ezeiru[1]

[1]*Catholic Relief Services, Abuja, Nigeria,* [2]*Management Sciences for Health (MSH), Abuja, Nigeria,* [3]*Malaria Consortium, Abuja, Nigeria,* [4]*Natonal Malaria Elimination Program, Abuja, Nigeria*

Nigeria account for 24% of the global malaria death and it remains the major causes of morbidity and mortality with pregnant women and children under five years. Evidence shows that early use of Insecticide-treated nets throughout pregnancy and in the postpartum period for both mother and child increases benefits. Despite the beneficial effect of sleeping under LLINs for the pregnant woman and her newborn infant, its uptake in routine health setting in Nigeria remain particularly poor. The proportion of pregnant women and under-five children who received LLINs health facility settings between July- December 2018 from data obtained from DHIS was 57% in the 13 Global Fund supported Catholic Relief Services (CRS) implementing states. To improve routine LLIN uptake in the health facility settings, a mix of strategies including maternal and child health, monitoring & evaluation and supply chain management was implemented over a period between July and December 2019. This include ensuring that program officers/states government officials undertake and participate in ANC/EPI clinics ensuring that all eligible pregnant women and children receive nets (Maternal and child health). Ensuring that all nets received are documented in the source document for reporting, as well as State M&E specialist to conduct monthly data review of LLIN uptake against eligible pregnant women and children (Monitoring and Evaluation) and ensuring that bimonthly Last Mile Distribution (LMD) of LLIN are implemented as scheduled and health facility LLIN stock are monitored to prevent stock outs (Supply Chain). Data obtained from DHIS shows average uptake of 80% in 7 states in a similar review period between July – December 2019. In the seven states LLIN uptake amongst pregnant women and under-five increased from 53.3% to 69.1 in Gombe state, 55.7% to 68.1% in Jigawa state, 58.2% to 81.3% in Adamawa state, 71.9% to 94% in Kwara state, 49.9% to 84.1% in Ogun state, 93.8% to 93.9% in Osun state and 60.9% to 67.2% in Taraba state. Implementation of the mix strategies contributed to overall program uptake and performance for LLIN amongst eligible pregnant women and children.

819

DEPLOYMENT OF MOBILE APPLICATION TO ENHANCE BREEDING SITE MAPPING AND CHARACTERIZATION FOR IMPROVED LARVICIDING IN OBUASI MUNICIPALITY, GHANA

Kwame Desewu, Nicholas Ato Egyir, Ignatius Williams, Samuel Asiedu, Rosemary Ampofo-Bekoe, Paul Osei Bonsu, Gilbert Dossah, Grace Oppong, Francis Amankwah, Joseph Mwinikubu

Anglogold Ashanti Malaria Control Limited, Obuasi, Ghana

The core interventions implemented against malaria vectors have been largely effective in reducing the burden of the disease, especially in sub-Saharan Africa. To further reduce malaria transmission and drive the cases down would require the introduction of additional measures. Larviciding is recommended as a complementary intervention to augment the core interventions such as indoor residual spraying (IRS) and long-lasting insecticide nets (LLINs). AngloGold Ashanti Malaria Control program has been implementing IRS project in Obuasi since 2006. Between August to December, 2017, AGAMal undertook larviciding with *Bacillus thurengiensis israelensis* (Bti) in the Obuasi area to complement its IRS activities. Potential breeding sites were geotagged and characterised using the SnooCODE® mobile application and mapped using Google Earth®. This contributed to a more precise quantification of personnel, larvicide and increased efficiency in tracing the sites for treatment and re-treatment. Entomological monitoring was done to assess juvenile and adult vector densities. There was a reduction in the larval and adult *Anopheles gambiae* s.l densities. It was shown that the larvicide was persistent and effective in the breeding sites for up to 7 days. The average pre-treatment and post-treatment densities for the early instars ranged from 20.5-34.9 larvae/10 dips/site to 0-3.8, respectively. Again, average pre-treatment and post-treatment densities for the late instars ranged from 27.9-42.5 larvae/10 dips/site to 0-3.9, respectively. August to December, 2017, recorded the highest total rainfall of 518.2mm, as compared to same period in 2015 (354.2mm) and 2016 (423.4mm). Even though this period in 2017 had the highest rainfall, the density of *An. gambiae* s.l was the lowest (n=1,831), compared to 3,097 and 3,212 in 2015 and 2016 respectively. A properly planned and executed larviciding has the potential to cause a significant decline in vector densities and malaria transmission in areas where core vector control tools have reduced and plateaued the number of malaria cases.

820

CHALLENGES OF IMPLEMENTING THE NATIONAL CHOLERA CONTROL PLAN IN BANGLADESH: REALISTIC ACHIEVEMENT WITH AVAILABLE RESOURCES

Firdausi Qadri

International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh

The Global Task Force on Cholera Control (GTFCC) has launched ambitious goals for reducing cholera deaths (90%) by 2030 and eliminating burden from different countries, including Bangladesh. Bangladesh has taken strong leadership for reporting cholera to the WHO and has endorsed the multi-sectorial National Cholera Control Plan (NCCP), which includes mass vaccination efforts and water/sanitation improvements (2019-2030). A massive influx of Rohingya (Forcibly Displaced Myanmar Nationals) from Rakhine State in Myanmar to Bangladesh has held in 2017 in response to internal conflict. The government of Bangladesh together with the support of icddr,b and partner organizations conducted six mass campaigns with OCV between October 2017-February 2020 in Cox's Bazar in this population. All campaigns were conducted in a very challenging situation which leads Bangladesh towards the roadmap of ending cholera 2030 agenda declared by GTFCC. This experience of MOH&FW, DGHS, and EPI was important for the implementation of OCV campaign in Dhaka. The first campaign was carried out in Mohammadpur, Adabor, Hazaribagh, Kamrangirchar, Darus-Salam, and Lalbagh in Dhaka for six days for 1.2 million doses of OCV. 24 million doses have been delivering as demonstration campaigns that have been planned in NCCP. Phase-wise vaccination will be carried out in cholera hotspots in Bangladesh. A total of 172.9 million of OCV doses will be required to align the NCCP for Bangladesh. The OCV supply needs to be increased to reduce the gap between the average number of doses approved and the number of doses ultimately shipped to the countries. Therefore, locally produce vaccines can be played a major role to overcome the constraints. Bangladesh has already produced first local OCV through technology transfer and licensed for Bangladesh (not WHO prequalified). If this vaccine can be used for the phase-wise vaccination campaign, the dose requirement from GTFCC may be reduced which will be helpful for the global roadmap.

821

ANTIBIOTIC RESISTANCE PROFILE OF BACTERIAL ISOLATES CAUSING DEATH OF CHILDREN UNDER FIVE YEARS OLD IN EASTERN ETHIOPIA

Mulu Berihun[1], Dadi Marami[1], Zelalem Teklemariam[1], Mussie Brhane[1], Mersan Deresa[1], Fami Ahmed[1], Nardos Assegid[1], Lola Madrid[2], Joseph Oundo[2], Nega Assefa[1]

[1]*College of Health and Medical Science, Haramaya University, Harar, Ethiopia,* [2]*London School of Hygiene & Tropical Medicine, London, United Kingdom*

Mortality in under five children is a global public health concern. Globally there were 5.6 million children deaths below five years in 2016. However, estimating their magnitude and antibiotic resistance profile, remains a challenge. This study was conducted from February 2019 and March 2020 at Child Health and Mortality Prevention Surveillance Ethiopia based at Hiwot Fana Specialized University Hospital and Kersa Demographic and Health Surveillance System. Total of 63 post-mortem specimens collected using Minimally Invasive Tissue Sampling techniques within 24hours of death. Bacterial isolation and identification was performed using standard microbiological methods. The antibiotics tested were gentamicin, ceftriaxone, clindamycin, erythromycin, penicillin, ampicillin, amoxicillin-clavulanic acid, trimethoprim-sulfamethoxazole and ciprofloxacin. Antibiotic resistant interpreted using 2018 Clinical and Laboratory Standards Institute guidelines. Definite cause of death of 53/63 cases was determined by synchronizing the laboratory result with verbal autopsy, demographic and clinical data abstraction through experts panel discussion. There were 21/53 and 3/53 cases with an infectious etiology as immediate and underlying cause of death respectively. A total of 15/24 were culture confirmed and the rest by TAC. From this 3/15 were >12 Month, 4/15 were >28 days, 3/15 were neonates, 3/15 early neonates and 2/15 were still birth. A total of 14(26.4%) deaths were due to sepsis with Klebsiella pneumoniae 6(40%), Non-Typhi Salmonella 3(20%),Escherichia coli2(13.3%),Pseudomonas aeruginosa1(6.7%), Staphylococcus aureus1(6.7%)and Streptococcus pyogenes1(6.7%)and one meningitis death due to Neisseria meningitidis. Multidrug resistance to two or more class of drugs was seen in 5/6 (83.3%) Klebsiella pneumoniae, 3/3 (100%) Non-Typhi Salmonella and 2/2 (100%) Escherichia coli. This work has demonstrated the importance of identification and antimicrobial resistance testing of bacterial pathogens. Additionally; demonstrated possible interventions and vaccine preventable infections in under five children.

822

REFUGEE SETTLEMENTS AND CHOLERA RISKS IN UGANDA DURING 2016-2019

Godfrey Bwire[1], Christopher Garimoi Orach[2], Freda Loy Aceng[1], Sam Emmanuel Arinaitwe[3], David Matseksese[4], Edson Tumusherure[5], Makumbi Issa[6], Allan Muruta[1], Rebecca D. Merill[7], Amanda K. Debes[8], Mohammad Ali[8], **David A. Sack**[8]

[1]*Ministry of Health, Department of Integrated Epidemiology, Surveillance and Public Health Emergencies, Kampala, Uganda,* [2]*Makerere University, College of Health Sciences, Uganda, Uganda,* [3]*Makerere University, College of Health Sciences, Kampala, Uganda,* [4]*UNICEF, Emergencies Programme, Kampala, Uganda,* [5]*Isingiro District Local Government, Department of Health, Isingiro, Uganda,* [6]*Ministry of Health, Emergency Operational Centre, Kampala, Uganda,* [7]*Centers for Disease Prevention and Control, Division of Global Migration and Quarantine, Atlanta, GA, United States,* [8]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States*

Cholera is one of the major health risks for refugees. Uganda hosts approximately 1.33 million refugees from neighboring countries. This study describes cholera outbreaks reported in refugee settlements in Uganda and proposes recommendations for prevention and control. Based on reported cholera cases from the Uganda Ministry of Health there were 1,495 cholera cases and 30 deaths from 2016 to 2019 occurring during seven outbreaks in five refugee settlements (Bidibidi, Pagirinya, Kyangwali, Kyaka II and Nakivale) and the Nyakabande reception centre. Kyangwali refugee settlement reported the largest number of cases, the highest attack rate and a high case fatality ratio (2.6%). Two outbreaks occurred in Kyaka II refugee settlement during two consecutive years (2018 and 2019). Outbreaks occurred concurrently during three of these years though the settlements were distant from each other. These outbreaks appeared to be initiated with the arrival of new groups of refugees. The outbreaks in the refugee settlements, in some cases, spread to the host community. Special interventions are needed to reduce the risk of cholera among incoming refugees, both to protect these vulnerable groups, but these interventions are also needed if Uganda is to reach cholera elimination as planned by 2030.

823

ORAL CHOLERA VACCINE TO PREVENT IMPENDING CHOLERA IN A HUMANITARIAN CRISIS: EFFECTIVENESS AND A SURVEILLANCE NETWORK IN BANGLADESH

Ashraful I. Khan[1], Md. Taufiqul Islam[1], Md. Mahbubur Rashid[1], Mokibul H. Afrad[1], Azimuddin Ahmed[1], Navid A. Tanvir[1], Minjoon Kim[2], ASM Mainul Hasan[2], Yasmin A. Begum[1], Maya Vandenent[2], Firdausi Qadri[1]

[1]*icddr,b, Dhaka, Bangladesh,* [2]*UNICEF, Dhaka, Bangladesh*

The large exodus of Forcibly Displaced Myanmar Nationals (FDMNs) to Cox's Bazar in Bangladesh led to a situation of humanitarian crisis since August 2017. To combat epidemics of cholera mass vaccination campaigns among refugees were conducted between 2017 and 2018 in four phases including the adjacent host population. A surveillance network was established in the catchment area to evaluate the effectiveness of an oral cholera vaccine among the unprivileged population. Facility-based diarrheal disease surveillance was conducted in Ukhiya and Teknaf Upazila of Cox's Bazar district from the end of 2017. Both FDMNs and Bangladeshi people being enrolled under this surveillance in 11 health facilities. On-site rapid diagnostic test (RDT) was carried out and specimens sent in transport medium to the Dhaka for microbiological culture. Descriptive analysis and vaccine effectiveness (VE) using the test-negative design (TND) was conducted. From September 19, 2017, to December 31, 2019, a total of 8,134 acute watery diarrheal patients were enrolled. Among them, 111 (1.4%) cases were found positive for *V. cholerae* by RDT whereas 72 (0.9%) by culture. Among all culture-confirmed cholera patients, 32 (44.4%) were from FDMNs and 40 (55.6%) from the host community. Among the diarrheal patients, a total of 2,564 patients received OCV. Overall, the protective efficacy of OCV among FDMN and the host community was 35% for all age groups (p- 0.118) and 79% among the 5 to 14-year age group (p- 0.034) and 67% among >15-year age group (p- 0.008). Moreover, cholera surveillance in the camp areas, NGO assisted health facilities and Upazila health complexes provided a keen visual on any sort of cholera upsurge and facilitate regular reporting to government, WHO, EWARS, UNICEF, IOM, and other related stakeholders to strengthen the response team to take immediate necessary measures. In conclusion, despite several challenges in evaluating vaccine effectiveness in this particular context, this study shows that timely vaccination and surveillance network prevent a major cholera outbreak in this humanitarian crisis condition.

824

TO DETERMINE DETECTION LIMIT OF REAL TIME POLYMERASE CHAIN REACTION FOR SALMONELLA TYPHI IN HUMAN BILE SAMPLES

Noshi Maria Fletcher, Mehreen Adnan, Sadaf Pervaiz, Junaid Iqbal, Farah Qamar

Aga Khan University, Karachi, Pakistan

Typhoid fever transmission is still an unconquerable problem in low- and middle-income countries due to gallbladder carriage of invasive Salmonella. To isolate S. Typhi from bile of patients who undergo cholecystectomy, culture method is still considered as a gold standard

method. But it is not free from limitations. Recent developments in molecular techniques like Polymerase Chain Reaction (PCR) are quite encouraging for rapid detection of Salmonella serovars with high sensitivity and specificity. Therefore, we aimed to determine detection limit of real time PCR for S. Typhi in bile samples. To attain our objective we performed colony forming unit assay and real time PCR. Our results showed that real time PCR can detect 10^2 CFU/ml at 35 Cq value that is 16 cells per reaction. Results of our study have provided useful data for researchers to set cutoff values of real time PCR for experiments. Our finding showed high sensitivity of real time PCR but detection limit of this technique should not be used ubiquitously as it vary according to type of specimen and methodology. Therefore, more studies are required to make it a technique of choice for various objectives in different fields.

825

EPIDEMIOLOGY OF CHOLERA CAUSED BY *VIBRIO CHOLERAE* SEROGROUP O139 ISOLATED IN BANGLADESH BETWEEN 2013 AND 2017

Md. Taufiqul Islam, Fahima Chowdhury, Yasmin Ara Begum, Ashraful I. Khan, Firdausi Qadri

International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

Cholera is a potentially epidemic and life-threatening secretory diarrhea caused by the organism *Vibrio cholerae* and manifested with numerous, voluminous watery stools, often accompanied by vomiting. More than 200 serogroups of *V. cholerae* have been identified. Among them, O1 and O139 are the most common serogroups which have been associated with epidemic cholera. *Vibrio cholerae* O139 was first identified in Bangladesh during 1992-1993 and then declined and have been sporadically reported since then. Descriptive analysis has been carried out on the 9 cases who were presented with acute watery diarrhea to icddr,b Mohakhali and Mirpur hospitals over the period 2013 to 2017 under hospital-based surveillance. Among them, 2 were admitted in 2013, 4 in 2014, and one each between 2015 to 2017. Clinical history was collected from the hospital database. During the hospital stay, fecal samples from all the cases were sent to the laboratory for bacteriological examination and found 9 cases *V. cholerae* serogroup O139. Among the nine cases, 22 %(n=2) were from <5years age group and rest from >18 years. 67% of patients were male whereas 33% female. The mean diarrheal duration of the patients was around 33 (SD±28) hours. All the patients had a history of passing loose watery stool. The nutritional status of all patients was normal. Around 67% of patients presented with vomiting and 11% suffered from fever. Four patients (44%) were infused with IV fluid. 22% of the patients presented with no sign of dehydration, 33% with some dehydration, and the rest 45% with severe dehydration. A total number of 7 cases had come from urban Dhaka, and 2 from outside the city (Savar and Munshiganj). These results suggest that *V. cholerae* O139 can cause clinical disease that persists in Bangladesh. Serogroup O139 probably exists in the environment and can cause disease that requires hospitalization. Even though outbreaks and epidemics have not been seen in the last 15 years since the emergence of this pathogen, it does retain the capacity to cause disease which emphasizes the necessity for continuing molecular epidemiologic surveillance of *V. cholerae* in Bangladesh.

826

STOOL SHEDDING OF *S.* TYPHI FROM TYPHOID FEVER PATIENTS AND ASYMPTOMATIC CHRONIC CARRIERS IDENTIFIED IN THE COMMUNITY BASED SURVEILLANCE CONDUCTED IN DHAKA, BANGLADESH

Farhana Khanam[1], Firdausi Qadri[1], John D Clemens[1], Andrew J Pollard[2]

[1]*icddr,b, dhaka, Bangladesh,* [2]*University of Oxford, Oxford, United Kingdom*

Typhoid fever is a serious public health concern in many resource poor countries, like Bangladesh. Transmission of *S*. Typhi is largely related to shedding from acute patients or asymptomatic carriers. Passive surveillance for typhoid fever was conducted a censused population. Blood and stool specimens were collected from febrile patients for culture. For the serosurvey component, an age-stratified cohort of over 8,000 individuals was randomly selected and blood was collected from them on day 0 and day 90 to measure anti-Vi antibody. Stool was collected from individuals with high anti-Vi antibody titre to investigate possible chronic stool carriage of *S*. Typhi. In the passive surveillance 332 *S*. Typhi bacteremic patients were identified among 6,305 suspected patients. Among them, 109 were also positive by stool culture at the day of enrolment. In addition, 33 patients were positive only by stool culture. Sixteen out of 290 patients were detected as convalescent carriers who had shown positivity for *S*. Typhi in their stool on day 30. Among 284 patients who agreed to give stool, 01 patient was temporary carrier, continued shedding the organism until day 180. On day 365, stool was collected from 16 patients who were excreting *S.* Typhi in stool either on day 30 or day 180, but none of them had shown positivity in stool culture. For the serosurvey, 303 participants had high Vi titre either on day 0 or day 90. Paired stool specimens 48 hours apart were collected from 192 participants who had high Vi after 6 months of enrolment and only one chronic, asymptomatic typhoid carrier was detected among them. Majority of typhoid fever patients was recovered from acute illness. Although some patients were continuing stool shedding during convalescent stage, but only one asymptomatic carrier was identified among the serosurvey participants. Hence the findings of the study reflect that the ongoing transmission of infection in the community is more likely by the acutely infected patients. The study illustrates the significance of improving water and sanitation infrastructure and introduction of typhoid conjugate vaccines to reduce the disease burden in Bangladesh.

827

ASSOCIATION OF TEMPERATURE, PRECIPITATION, AND HUMIDITY WITH INCIDENCE OF INFECTIOUS DIARRHEAL DISEASE IN THE SICHUAN PROVINCE OF CHINA FROM 2005 TO 2016

Kelly McCain[1], Elizabeth Sajewski[1], Qu Cheng[2], Justin Remais[3], Changong Yang[4], Song Liang[5], Benjamin Lopman[1]

[1]*Rollins School of Public Health, Emory University, Atlanta, GA, United States,* [2]*University of California - Berkeley, Berkeley, CA, United States,* [3]*University of California, Berkeley, Berkeley, CA, United States,* [4]*Institute of Health Informatics, Sichuan Center for Disease Control and Prevention, Chengdu, China,* [5]*Department of Environmental and Global Health College of Public Health and Health Professions, University of Florida, Gainesville, FL, United States*

Infectious diarrhea is a major cause of morbidity and mortality globally, causing 1.6 million deaths and 49.8 million disability-adjusted life years lost in 2016. Diarrheal disease often has distinct seasonality, with viral cases more often occurring in the colder, drier winter months and bacterial cases more often in the warmer, wetter summer months. In Sichuan, China, and the capital city of Chengdu, while there has been an overall decreasing trend in diarrheal disease, seasonal patterns have shifted from annual to biannual peaks, beginning in January 2012. This study utilized China's National Infectious Disease Reporting System to investigate the relationship between meteorological factors and diarrheal disease and how the shift in disease seasonality modified the impact of temperature, humidity, and precipitation on the incidence of 'other infectious diarrhea' in Chengdu. A negative binomial generalized linear model was fit to estimate the effects of extreme precipitation, relative humidity, and temperature on diarrheal disease, while controlling for season and autocorrelation. Relative humidity lagged 1 week was associated with diarrheal disease (IRR, 95% CI: 0.995 (0.990, 0.999) for each 1% increase in relative humidity). No significant relationships were found between all other meteorological terms and diarrheal disease either before or after the shift to biannual peaks. The weekly rate of diarrheal disease after the shift in 2012 was 3 times higher than the rate beforehand (IRR, 95% CI: 3.04 (1.09, 8.46)). The significance of the diarrheal disease autocorrelation terms, the log of the count of cases lagged at 1-4 weeks, indicates that disease prevalence is more

strongly associated with transmission of diarrheal disease in Chengdu than meteorological factors (IRR, 95% CI: 1-week lag: 1.20 (1.13,1.28); 2-week lag: 1.16 (1.09, 1.24); 3-week lag: 1.10 (1.03, 1.18); 4-week lag: 1.07 (1.00, 1.14) for each additional case). Further research that includes both infectious disease dynamics and meteorological factors is needed to identify other drivers may have contributed to the shift in seasonality of diarrheal disease cases.

828

HEALTHCARE UTILIZATION SURVEY AND ITS RELEVANCE IN THE HYBRID SURVEILLANCE MODEL-FINDINGS OF THE STUDY CONDUCTED BY THE SURVEILLANCE OF ENTERIC FEVER IN INDIA (SEFI) NETWORK

Reshma Raju[1], Kezia J. Angelin[1], Dilesh Kumar[1], Ranjith R. Kumar[1], Nikhil Sahai[1], Karthik Ramanujam[1], Manoj Murhekar[2], A. Elangovan[3], Prasanna P. Samuel[1], Jacob John[1], Gagandeep Kang[1]

[1]*Christian Medical College Vellore, Vellore, India,* [2]*ICMR-National Institute od Epidemiology, Chennai, India,* [3]*ICMR-National Institute of Epidemiology, Chennai, India*

Enteric fever is a major public health challenge in low/middle-income countries. As reliable incidence data was lacking in India, Surveillance of Enteric Fever in India(SEFI) network was set up in 2017. Of the18 sites where the enteric fever surveillance was performed, 6 utilized a hybrid surveillance model, a combination of a facility based surveillance to determine the crude incidence of typhoid, and community based healthcare utilization, to derive the adjustment factor, which when applied to the crude incidence, gives an accurate estimate of the true burden of disease. The facility-based surveillance was conducted in 6 secondary hospitals. The methodology and results of the community-based healthcare utilization survey is described here. 5000 households were surveyed from each site using a two-stage sampling process. Demographic data and healthcare-seeking behaviour were assessed in the sampled households based on a 2 week and 12-month recall. A total of 137,990 subjects in 30,308 households were surveyed. Hospitalization episodes from any cause ranged from 20/1000 in Himachal Pradesh to 47/1000 in Andhra Pradesh. Of these, febrile hospitalization episodes ranged from 2.5/1000 in Himachal Pradesh to 9.6/1000 in Andhra Pradesh. 23% of all febrile admissions were among the children less than 15 years. The median duration of hospitalization was 4 days. The percentage of febrile admission that sought care in the sentinel facility varied across sites and was found to be 17% in Andhra Pradesh, 38.3% in Chandigarh, 9.6% in Maharashtra, 37.5% in Assam, 35.7% in Himachal Pradesh and 23.5% in Bihar. These percentages are lower than the presumed 60%, taken at the time of choosing the facilities for the hybrid surveillance. The variability in healthcare utilization for fever admissions in the sentinel hospitals underscores the importance of performing periodic healthcare utilization surveys, as a large proportion of people seek other sources of healthcare. Hence it is important to adjust for those cases which have slipped out of the facility surveillance radar, in order to obtain a true estimate of the burden of typhoid fever.

829

PRELIMINARY FINDINGS IN CARBAPENEM RESISTANT ENTEROBACTERIACEAE INCIDENCE AT A TERTIARY HOSPITAL IN RAJSHAHI, BANGLADESH

Abdullah Awsaf Noor[1], Sabera Gul Nahar[2], Sanjida Jahan[3], Obinna Nnedu[1]

[1]*Ochsner Health System, Jefferson, LA, United States,* [2]*Rajshahi Medical College Hospital, Rajshahi, Bangladesh,* [3]*Ochsner Health System, New Orleans, LA, United States*

Carbapenem resistant enterobacteriaceae (CRE) has emerged as a major public health concern in low and middle income countries like Bangladesh. World Health Organization (WHO) has identified research gaps and the need for additional well-designed studies in these countries. This study provides preliminary information on CRE incidence at a tertiary hospital in Rajshahi, Bangladesh. Retrospective chart review followed by prospective data was collected to document CRE and CSE incidence at Rajshahi Medical College Hospital Laboratory from March through May 2018, and February 2019 respectively using disc diffusion method. Resistance was identified using CLSI standards. The retrospective chart review (3/1/2018-5/31/2018) showed 56 *Escherichia.Coli* (*E coli*), 8 *Klebsiella pneumoniae* (*K pneumoniae),* and no Enterobacter sp isolates during the 3-month period. Of the *E.Coli* tested isolates, 67% were imipenem resistant, and 15% were meropenem resistant. Of the *K pneumoniae* tested isolates, 80% of them were imipenem resistant, and 0% were meropenem resistant. The prospective data collection (2/1/2019-2/28/2019) documented incidence of 19 *E.Coli*, 1 *K pneumoniae* isolates, and no *Enterobacter sp*. Among E. Coli strains, 42% in urinary cultures and 100 % in wound cultures (surgery or trauma) were resistant to meropenem, and 16% in urinary cultures and 33% in patients undergoing wound culture were resistant to imipenem. Klebsiella was sensitive to both imipenem and meropenem. Our data showed higher incidence of imipenem resistant organisms than that found at a tertiary hospital in Dhaka, Bangladesh, but similar to that found in Maharastra, India. The preliminary data shows a considerable resistant pattern. Research design including confirmatory testing and genetic characterization will better inform about CRE prevalence in the hospital.

830

DIAGNOSIS OF HEPATIC HYDATID DISEASE PRESENTED WITH SOFT TISSUE DENSITY LESION AND MIMICKED MALIGNANCY BY FINE NEEDLE ASPIRATION CYTOLOGY

Shankar Kafle, Purbesh Adhikari, Sapana Sedhain, Bandana Mudbhari

B.P Koirala Institute of Health Sciences, Dharan, Nepal

Hydatid disease (Echinococcosis) is caused by a parasite of genus Echinococcus at a larval stage. It primarily affects the liver which usually presents with well-known characteristic imaging findings. Ultrasonography (USG) and Computerized Tomography (CT) are most useful in establishing diagnosis of hydatid disease. CT is more sensitive and accurate compared to USG. However, with progressively increasing size of cyst, the disease may mimic gross ascites or liver tumor. Fine Needle Aspiration Cytology (FNAC) has been used in the preoperative diagnosis of hydatid disease since 1980's by demonstration of hooklets and scolices in the aspiration fluid. We herein describe the cytologic features of a rare case of hepatic hydatid disease presented in a 63-year-old female situated in the right sub hepatic and gall bladder fossa region, clinically and radiologically suspicious for malignancy.

831

TAENIA ASIATICA CYSTICERCOSIS IN PIGS IN PHU THO PROVINCE, NORTHERN VIETNAM

Man Thi Nguyen[1], Pierre Dorny[2], Veronique Demauw[2], Thanh Giang Nguyen[3], Anke Van Hul[2], Chien hong Nguyen[4]

[1]*National Center for Veterinary Diagnosis, Hanoi, Viet Nam,* [2]*Institute of Tropical Medicine, Antwerp, Belgium,* [3]*National Institute of Veterinary Research, Hanoi, Viet Nam,* [4]*National University of Agriculture, Hanoi, Viet Nam*

Taenia asiatica, a zoonotic parasite, has been confirmed to exist in humans (i.e. its final host) in Vietnam, but its presence had not been investigated in pigs (i.e. its intermediate host) in the country. The aim of the study was to confirm the occurrence of *Taenia asiatica* in pigs in Vietnam by molecular technique. From July to August 2018, a total of 399 pig livers were sampled in 51 slaughterhouses in six communes in Phu Tho, a mountainous province in Northeast Vietnam. Suspected cysticerci and spots were collected and the DNA was extracted after which the species was identified by PCR-RFLP of a mitochondrial 12S rDNA fragment, and confirmed by sequencing. The result shows that no suspected *T. asiatica* cysts in collected livers detected (i.e. small milky white bladder with an invaginated head, on the liver surface or in the parenchyma). However, suspected lesions (small white solid spots) found in the liver of two pigs

presented with the 4 bands (100, 131, 190, 309 bp) of *T. asiatica* in the PCR-RFLP technique; and these results were confirmed by sequencing. These pigs were slaughtered in different slaughterhouses of the same commune. The prevalence of *T. asiatica* in Phu Tho was estimated at 0.50% (Exact CI: 0.06-1.80%). This is the first study to confirm the occurrence of *T. asiatica* in pig livers and justify the complete life cycle of this parasite in Vietnam. Considering that the *T. asiatica* cysticerci found in the liver were small, the prevalence of this parasite could be underestimated using morphological identification.

833

HEALTHY SUBJECTS IN *TAENIA SOLIUM* ENDEMIC REGIONS EXHIBIT NEUROCYSTICERCOSIS ASSOCIATED INNATE IMMUNE GENE EXPRESSION

Vasudevan Prabhakaran[1], Subashini Thamizhmaran[1], Susan Yang[2], Betcy Evangeline[1], Justin Josephin[1], Ravi Anupriya[1], Miao Zhang[3], Hélène Carabin[4], Douglas A. Drevets[3], Vedantam Rajshekhar[1], Ranjith Moorthy[1], Anna Oommen[5]

[1]*Dept. Of Neurological Sciences, Christian Medical College, Vellore, Tamilnadu, India,* [2]*Trinity College, Dublin, Ireland,* [3]*Dept. of Internal Medicine, University of Oklahoma HSC, Oklahoma, OK, United States,* [4]*Faculté de Médecine Vétérinaire and École de Santé Publique, Université de Montréal and Centre de Recherche en Santé Publique, Québec, QC, Canada,* [5]*Gudalur Adivasi Hospital, Gudalur, Tamilnadu, India*

Taenia solium is endemic to India where at least 1/1000 population is affected by the parasitic cyst infection of the brain (neurocysticercosis, NCC). We have earlier shown that monocytes from patients with NCC-associated seizures differentially express a variety of genes when compared with monocytes from patients with epilepsy of unknown aetiology (EUE). This study examines the degree to which genes identified in the context of NCC-associated seizures are differentially expressed when monocytes from healthy Indian subjects are exposed to *T. solium* antigens and *Mycobacterium tuberculosis* antigens (another infectious disease that is endemic to India). CD14+ monocytes isolated from 12 healthy Indian volunteers were exposed to 10 µg/ml of *T. solium* antigen, Bacille Calmette-Guérin (BCG) or purified protein derivative (PPD) for 4, 6, 12 and 24 hours and 1 µg/ml of LPS for 24 hours. RNA extracted from the cells was studied by RT-PCR for expression of the following 14 genes with higher expression among NCC patients compared to patients with EUE: GBP1P1, GBP1, CHN2, RAP1A, PLCG2, TAGAP (GTP related genes), LRRF1P2, IL20RB, TAX1BP1 (immune related genes), FEZ2 and TOR3A (neural processing genes) and MZB1, PECAM1, SLC8A1 (miscellaneous genes). Expressions of the pro-inflammatory cytokines, TNF-α, IL-1β, and IFN-1β were also studied. The Mann Whitney tests ($p<0.05$) were used for significance difference. *T. solium* antigen stimulation significantly upregulated TAX1BP1, SLC8A1 and TAGAP between 4 and 24 hours compared to BCG and PPD. Expressions of RAP1A, GBP1P1, GBP1, PLCG2, PECAM1, CHN2 and pro-inflammatory cytokine IL-1β were significantly upregulated by *T. solium* antigen stimulation at varying time points compared to PPD. These results show that genes upregulated in patients with NCC-associated epilepsy are also upregulated in monocytes of healthy donors from infection endemic regions, upon stimulation with *T. solium* antigens, to a greater extent than in response to mycobacterial antigens. This was not a generalized trend as this difference was not noted in expression of the 2 of 3 cytokine genes.

834

SERUM PROTEINS IDENTIFIED BY MASS SPECTROMETRY ARE POTENTIAL BIOMARKERS OF NEUROCYSTICERCOSIS ASSOCIATED EPILEPSY

Hélène Carabin[1], Betcy Evangeline[2], Chhaya Patole[3], Subashini Thamizhmaran[4], Josephin Justin[5], R. Ravi Anuiya[5], Miao Zhang[6], Douglas A. Drevets[6], Vedantam Rajshekhar[5], Ranjith Moorthy[5], Vasudevan Prabhakaran[5], Anna Oommen[7]

[1]*Faculté de Médecine Vétérinaire-Université de Montréal, Montreal, QC, Canada,* [2]*Dept. Of Neurological Sciences, Christian Medical College, Vellore, India,* [3]*Proteomic Division, Mass Spectrometry Facility, NCBS-TIFR, Bangalore, Karnataka, India,* [4]*Dept of Neurological science ,Christian Medical College, Vellore, India,* [5]*Dept. Of Neurological Sciences, Christian Medical College, Vellore, India,* [6]*Dept. of Internal Medicine, University of Oklahoma HSC, Oklahoma City, OK, United States,* [7]*Gudalur Adivasi Hospital, Gudalur, India*

Seizures are common to many brain disorders and their causes need to be determined for optimal treatment. This is exemplified by neurocysticercosis (NCC), *Taenia solium* larval infections of the brain, the most common cause seizures affecting at least 1/1000 persons in India between 2 and 60 years of age. Brain imaging is important in the diagnostic evaluation of seizure disorders but is inaccessible for many people living in LMIC. Brain imaging might be avoidable in some cases if blood biomarkers could identify the cause of seizures. Previous work from our group has shown that electrospray ionization MS (ESI-MS) serum profiles can discriminate between patients with NCC-associated epilepsy and those with epilepsy of unknown etiology (EUE). The present study assess if ESI-MS/MS of serum tryptic digests can differentiate epilepsies associated with NCC from those associated with mycobacterial infections and gliomas. Tryptic digests of sera from patients with solitary cysticercus granulomas, multi-lesion NCC, single cyst calcifications, tuberculoma, glioma, EUE (8 in each group) and 7 control subjects with no seizures and negative brain imaging, were subject to reverse-phase liquid chromatography followed by ESI-MS/MS. Mass peaks from 375 to 1950m/z were compared between the study groups. Proteins of mass peaks associated with each group were identified with MASCOT software and the human proteome database from Uniprot at 5% false discovery rate and a requirement for 2 unique peptides / protein. Four hundred and seventy eight proteins were identified in patients with NCC, 239 in those with EUE, 238 in those with tuberculomas, 237 in those with gliomas and 210 in controls. After exclusion of proteins identified in controls, Venn diagrams revealed that 140 proteins were specifically associated with NCC and were not seen in sera of patients with glioma, tuberculoma and EUE. A large number of serum proteins are unique to patients with NCC associated epilepsy that are not seen in sera of patients with epilepsies associated with brain mycobacterial infections and tumors and may have utility as biomarkers for NCC.

835

RECOGNIZING DISSEMINATED CYSTICERCOSIS IN THE TROPICS: A CASE REPORT

Jessica Tuan[1], Leway Kailani[2], Protogene Ngabitsinze[3], Sylvie Umuganwa[3], Faustin Munyaneza[3], Emile Musoni[3], Alvaro Laga Canales[4], Menelas Nkeshimana[5]

[1]*Yale University School of Medicine, New Haven, CT, United States,* [2]*Geisel School of Medicine at Dartmouth, Hanover, NH, United States,* [3]*University of Rwanda College of Medicine and Health Sciences, Kigali, Rwanda,* [4]*Brigham and Women's Hospital, Boston, MA, United States,* [5]*University Teaching Hospital of Kigali, Kigali, Rwanda*

We describe a 46-year-old Rwandan woman from Kamonyi District, in the southern province of Rwanda, who presented with a two-week history of bilateral lower limb weakness, causing difficulty walking. She had associated fevers and headache. She was febrile and tachycardic, with decreased lower extremity strength and subcutaneous nodules on her trunk and extremities. Laboratory data demonstrated leukocytosis with neutrophilic predominance and mild eosinophilia. Excisional biopsy

of a subcutaneous nodule revealed a cyst containing a protoscolex with suckers, ramifying cistern, and calcareous bodies; brain magnetic resonance imaging demonstrated diffuse, cystic cerebral, cerebellar, and soft tissue lesions—consistent with disseminated cysticercosis. She was treated with 30 days of albendazole and prednisolone, and afterwards, noted restored ability to walk independently. Disseminated cysticercosis is a rare complication of cysticercosis, and limited case reports describe such complications in Rwanda. Per literature review, seroprevalence of human cysticercosis in Rwanda has been reported at approximately 7 percent. Prior literature reports that there is an increased prevalence of cysticercosis in people with epilepsy in the southern province of Rwanda, and that approximately 21 percent of patients with epilepsy in southern Rwanda were positive for cysticercosis immunoblot testing which detect glycoproteins of *Taenia solium*. Cysticercosis is a parasitic disease, caused by larval cysts of the *Taenia solium* tapeworm. Disseminated cysticercosis occurs when *Taenia solium* embryos disseminate from the hepatoportal system to other organ systems and tissues in the body. This patient presented with an atypical presentation of cysticercosis, with manifestations involving the skin, skeletal muscles, soft tissues, and brain. Thus, clinicians in cysticercosis-endemic regions, such as Rwanda, should keep disseminated cysticercosis on the differential diagnosis with such atypical clinical manifestations, in order to recognize and promptly treat affected patients.

836

COMPARISON OF MONOCYTE GENE EXPRESSIONS IN RESPONSE TO *TAENIA SOLIUM* ANTIGENS IN HEALTHY SUBJECTS BETWEEN ENDEMIC AND NON-ENDEMIC REGIONS OF NEUROCYSTICERCOSIS

Miao Zhang[1], Subashini Thamizh[2], Vasudevan Prabhakaran[2], Betcy Evangeline[2], Justin Josephin[2], Ravi Anupiya[2], Ranjith Moorthy[2], Anna Oommen[2], Vedantam Rajshekhar[2], Helène Carabin[3], Douglas A. Drevets[1]

[1]*University of Oklahoma Health Sciences Center, Oklahoma City, OK, United States,* [2]*Christian Medical College Vellore, Vellore, India,* [3]*Université de Montréal, Montréal, QC, Canada*

Neurocysticercosis (NCC) is a parasitic zoonosis caused by larvae of *Taenia solium* and is responsible for up to one third of epilepsy in endemic areas. Our preliminary studies identified 14 monocyte genes upregulated among NCC-associated epilepsy patients compared to patients with epilepsy of unknown etiology. Our study aimed at evaluating if these genes are regulated by direct exposure to *T. solium* antigens and if monocytes from donors of NCC endemic and non-endemic regions respond differently. This was accomplished by isolating $CD14^+$ monocytes from nine healthy Indian (NCC endemic) and 11 US (non-endemic) donors, then incubating the cells with 1 ug/ml *T. solium* metacestode lentil lectin-purified glycoproteins (TsGP) for 4h to 24h. Expressions of 12 candidate genes, as well as pro-inflammatory genes were measured by qPCR. In monocytes from Indian donors, TsGP stimulation significantly upregulated 5 of 12 genes (GBP1, RAP1A, CHN2, FEZ2, MZB1) over time, in contrast to only 2 genes (GBP1 and LRRFIP2) in US donors. Expression of IL-1β was significantly increased in both groups, but to a significantly greater degree in US donors at 4h following TsGP stimulation. TNF was increased in US donors at 4h and 6h, but decreased in India donors at 4h before increasing at 24h. To test whether the difference in monocyte gene expressions between Indian and US donors is due to trained innate immunity, monocytes from US donors were trained by 10 µg/ml TsGP for 24 h, and then rested for 3 days before re-stimulation by 10 µg/ml TsGP, or 10 ng/ml LPS for 6h. A modest training effect was identified that decreased expressions of pro-inflammatory (TNF, IL1β and IL6) genes after re-stimulation with TsGP and LPS. Thus, TsGP induces monocyte gene expression differentially between healthy donors from NCC endemic and non-endemic regions. Monocytes trained by TsGP tend to suppress pro-inflammatory cytokine gene expression. The training effects may contribute to gene expression differences in monocytes between NCC endemic and non-endemic regions.

837

USE OF CYSTICERCOSIS CIRCULATING ANTIGEN IN PATIENTS WITH NEUROCYSTICERCOSIS

Cesar Gabriel Berto[1], Prasanth Balasubramanian[1], John Noh[2], Christina Coyle[3]

[1]*Jacobi Medical Center, Bronx, NY, United States,* [2]*Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention (CDC), Atlanta, GA, United States,* [3]*Albert Einstein College of Medicine, Bronx, NY, United States*

Detection of circulating parasitic antigens by ELISA for neurocysticercosis (NCC) are only present in the serum of patients with viable parasitic tissue; serum concentrations decrease with effective therapy. With high parasite burden, such as in subarachnoid neurocysticercosis (SANCC), cysticercus antigen detection is useful to assess response to therapy. A retrospective report of patients with neurocysticercosis who presented to Jacobi Medical Center in New York City is presented. The cysticercosis antigen by ELISA was measured in serum and cerebrospinal fluid (CSF), if available, at the Centers for Disease Control and Prevention. A total of 172 patients were included; 110 patients (64%) were male and the mean age was 37.0 ± 13.2. Disease involved parenchyma alone in 81(47.1%) patients followed by parenchymal and subarachnoid in 32 (18.6%) patients, only SANCC in 31(18.0%) patients, and intraventricular alone in 9 (5.2%) patients. The cysticercus antigen in serum was positive in 38 patients (22.5%); of these, 37 had extraparenchymal NCC. All patients were treated until resolution or stabilization of imaging and negative antigen levels detected, except in three patients with persistent antigen in serum or CSF. All of these three experienced a relapse of disease after discontinuation of therapy. The presence of antigen in serum and CSF correlated with extra-parenchymal involvement in NCC. The sensitivity of this test for parenchymal NCC was poor as reported in the literature. A positive circulating antigen at the end of treatment predicted relapse.

838

POSTNATAL CARE QUALITY IN RURAL ZAMBIA: PROVIDERS' AND USERS' PERSPECTIVES

Cephas Sialubanje[1], Thandiwe Ngoma[2], Lawrence Mwananyanda[2], Jeanette Kaiser[3], Rachel Fong[3], Davidson H. Hamer[3], Nancy Scott[3]

[1]*Levy Mwanawasa Medical University, Lusaka, Zambia,* [2]*Right to Care, Lusaka, Zambia,* [3]*Boston University, School of Public Health, Boston, MA, United States*

Postnatal care (PNC) is an important set of services offered to women and their babies to prevent maternal and newborn complications and deaths. This qualitative study aimed to explore user and provider perspectives on PNC services and factors affecting the quality of PNC services in Zambia. Forty focus group discussions (FGDs) (n=415 participants) and twelve in-depth interviews (IDIs) were conducted in four districts from Southern and Eastern provinces. FGDs comprised women who delivered within the last year, fathers, community elders and volunteers. IDIs comprised health workers at facility, district and provincial levels. Perspectives on quality of PNC services and factors affecting its quality were explored. Data were analysed using content analysis guided by the international quality of care domains derived from the World Health Organisation quality of care framework. Findings were triangulated among respondent types to elicit similarities and differences in perceptions of PNC quality and contributing factors. Most district and provincial levels providers perceived services to be of good quality due to availability and dissemination of new PNC guidelines. Nevertheless, providers from health facilities, service users and community volunteers described services to be of low quality due to limited staff training on new guidelines, low supply of essential emergency obstetric and newborn equipment, medicines, commodities, and small examination rooms providing inadequate privacy. Other reasons were low staffing levels, long waiting time, negative staff attitudes, low levels of confidentiality and limited transport for referral of obstetric emergencies and complications. Women who felt mistreated or perceived low quality

of services during pregnancy end delivering from home, do not return or start PNC visits late. Our findings suggest a need for interventions to improve PNC service quality through improved supply of emergency equipment, medicines and commodities, increasing staffing levels, privacy and confidentiality, shortening waiting time, improving referral services, user and provider perceptions of quality

839

EXPERIMENTAL CHOLERA DIARRHEA IN NORTH AMERICAN VOLUNTEERS

James M. McCarty[1], Lisa Bedell[2]

[1]*Stanford University, Stanford, CA, United States,* [2]*Emergent BioSolutions, Gaithersburg, MD, United States*

Background: *Vibrio cholerae* may cause severe and potentially life-threatening diarrhea in travelers. Methods: To assess the potential impact of cholera diarrhea in travelers, daily stool output after a cholera challenge in healthy adults aged 18-45 years was analyzed. Following gastric acid neutralization, volunteers ingested 1 x 10^5 colony forming units (CFU) of virulent *V. cholerae* O1 El Tor Inaba strain N16961 and were monitored for 10 days. The number and volume of diarrheal stools were measured in 24-hour intervals. Subjects were treated with oral or intravenous hydration as needed and a 5-day course of oral ciprofloxacin was initiated on day 4 after ingestion or if cumulative diarrhea reached 5.0 liters. Results: A total of 66 subjects were challenged with *V. cholerae* and diarrhea developed in 93.4%. Mean daily stool volume peaked at 1.972 liters per 24-hour period on post-challenge day 3 and was as high as 13.231 liters per day in one subject. The mean overall stool volume was 4.524 liters with one subject having 24.374 liters total. The mean daily number of diarrheal stools peaked at 8.1 per 24-hour period on day 3 with one subject having 29 stools in a day. The mean overall stool number was 24 with one subject having a total of 79 diarrheal stools. Diarrhea lasted 5 or more days in 56% of subjects. Conclusion: In a controlled setting, *Vibrio cholerae* infection results in diarrhea which may be severe and include massive fluid loss. Cholera may be potentially life threatening in travelers, particularly those without rapid access to high quality medical care and rehydration. Vaccination against cholera is an important part of prevention when traveling to areas of endemic disease.

840

ATYPICAL MENINGOCOCCAL PURPURA FULMINANS ABOUT TWO CASES OBSERVED IN ADULTS AT THE HOSPITAL OF THE SINO-CONGOLESE FRIENDSHIP OF N'DJILI IN KINSHASA (DRC)

Hassan Kamena Mwana-Yile

Hospital of the Sino-Congolese Friendship of N'djili, Kinshasa, Democratic Republic of the Congo

Hassan Mwana-Yile [1], Nyembue Viviane [1], Mayalale Héritier [1], Ngoie Divita [1], Kamasaki Christelle[1], Bala Mike[1], Situakibanza Hippolyte [21] Departement of Internal Medicine, Hospital of Sino-Congolese Friendship, Kinshasa.[2] Departments of Internal Medicine and Tropical Medicine, Faculty of Medicine, University ofKinshasa

Meningococcal meningitis is a public health problem in the African meningitis belt but also in the Democratic Republic of Congo. This study was undertaken to evaluate cases of meningococcal purpura fulminans observed in a hospital in Kinshasa in two adults (DRC). Cases Two patients aged 36 and 53 years old, living respectively in N'djili and Masina squares, were admitted in coma stage III (Glasgow 6/15) with deviation of the mouth from the side left and bruising. The cerebrospinal fluid was unremarkable. Blood culture reveals a strain of *Neisseria meningitidis* in both patients sensitive to all of the antibiotics tested. Antibiotic therapy based on C3G was initiated as a matter of urgency. The evolution was marked by death in a table of MODS. Our observations encourage us to be vigilant in the face of febrile purpura and should evoke meningococcal meningitis despite the fact that the country is not classically counted in the African meningitides belt.

841

DIARRHEIC DISEASES IN KINSHASA / DRC

Sandra Mvumbi,M. G Musungayi

PNLCD, Kinshasa, Democratic Republic of the Congo

Over 1.7 billion cases worldwide, 2nd cause of death in children inferior 5 years old, One of the main causes of malnutrition in children inferior 5 years old 3rd reason for pediatric consultation in Endemo-epidemic mode. This study was undertaken to 1) assess the situation of diarrheal diseases in the city of Kinshasa province and the institutional capacity in the response to the problem. 2) describe the spatio-temporal distribution of diarrheal diseases in 2018, 3) identify the explanatory factors for this morbid phenomenon; and 4) evaluate the institutional capacity in the operational response to the phenomenon. A retrospective study was carried out on 10 health zones of the City province of Kinshasa the data were collected on registers of health facilities, documentary review and household survey. Data were organized in Excel and processed on sphinx. Evolution under an endemic mode persistence with upward trend. Either 75.4 percent in the west and 73.7 percent in the east, The degradation of wells, inability of the regideso to adequately supply drinking water throughout the city Kinshasa, the use of unhygienic toilets increases morbidity, The runoff of water following the slope and the dynamics of land use favor the increase of morbidity due to diarrheal diseases. In conclusion, persistence of diarrheal diseases with increasing trend following environmental vulnerability, Low availability of inputs, less trained human resources and non-operational community dynamics favor the weak institutional response

842

IMPACT OF THE INTRODUCTION OF PCV7/13 ON ANTIMICROBIAL RESISTANCE IN INVASIVE PNEUMOCOCCAL DISEASE IN INDONESIA

Mam Mass Sey, Omar Samba, Mustapha Danso, Dawda Kijera, Abdou Barrow

State University Syarif Hidayatullah Jakarta, Jakarta, Indonesia

We describe antimicrobial resistance in invasive pneumococcal disease due to all serotypes and non-vaccine types (NVT) pre and post pneumococcal conjugate vaccine (PCV) implementation in Indonesia in all age groups. We identified and performed antimicrobial susceptibility testing using disc diffusion methods on pneumococcal isolates obtained from invasive samples collected from standardised population-based pneumococcal disease surveillance in the Pamulang Health Surveillance System. The study began July 2010. PCV7 was presented in August 2011 & PCV13 in June 2013. Antibiotic susceptibility was interpreted using Standard Laboratory guidelines. We plotted case counts of invasive pneumococcal disease in the pre-PCV, PCV- introduction, and post-PCV13 implementation period. 450 pneumococcal isolates were screened against five antimicrobial agents. There was a decline in antibiotic resistance in all age groups in invasive pneumococcal disease during vaccine implementation. In the 2-23 month age group, annual counts of oxacillin, chloramphenicol, and tetracycline resistant cases fell from 10-15 in 2011 and 2012 to 6-7 in 2015 and 2016. In the 24 - 59 month age group, there was a decrease in tetracycline resistant cases. In those >5 years, oxacillin, chloramphenicol, and tetracycline fell to zero cases in 2014 and 2015. Resistance fell due to reductions in vaccine-serotypes 1, 5,14 and 23F. The resistantance of NVT cases increased over time, mainly in the 2-23 month age group, with tetracycline resistance mainly in serotype 10A, 12F, 11B, 7C and 25A and tetracycline resistance in serotype 12F in 2017. Isolates were sensitive to erythromycin but 95-98% was resistant to cotrimoxazole. In conclusion, there is an overall reduction in cases of antimicrobial resistant IPD, resistance is emerging in NVT. We hypothesise that increased transmission of NVT after the introduction of PCV and exposure to antimicrobials

facilitates the emergence of resistance in NVT. Ongoing surveillance is important to determine future trends in resistance as it has both clinical and public health importance in PCV era.

843

ACCURACY AND RELIABILITY OF FOCUSED ECHOCARDIOGRAPHY IN PATIENTS WITH CHAGAS DISEASE FROM ENDEMIC AREAS: SAMI-TROP COHORT STUDY

Maria Do Carmo P. Nunes[1], Isabella Barros[1], Marcio Barros[1], Antonio Antonio[1], Raul Silva Camargo[1], Claudia Oliveira[2], Ariela Ferreira[3], Lea Campos Oliveira[4], Ana Luiza Bierrenbach[4], Desireé Haikal[5], Larissa Martins[1], Clareci Cardoso[2], Ester Sabino[6]

[1]*Universidade Federal de Minas Gerais, Belo Horizonte, Brazil,* [2]*Federal University of São João del-Rei, Divinópolis, Brazil,* [3]*Health Science Program, Universidade Estadual de Montes Claros, Montes Claros, Brazil,* [4]*Hospital das Clinicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil,* [5]*Universidade Estadual de Montes Claros, Montes Claros, Brazil,* [6]*Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil*

Chagas disease remains a major cause of cardiovascular death in endemic areas. Focused echocardiography (FoCUS) is a point-of-care means of assessing cardiac function which can be useful for the diagnosis of cardiac involvement. This study aims evaluating the characteristics of validity and reliability of FoCUS applied on Chagas disease patients. Patients with Chagas disease coming from an endemic area were selected from a large cohort (SaMi-Trop). A simplified echocardiogram with only three images was extracted from the conventional full echocardiogram performed in this cohort. The images were evaluated by an observer who was blinded to the clinical and echocardiographic data, to determine the accuracy and reliability of FoCUS for cardiac assessment. The analysis constituted of 5 prespecified image elements, dichotomized in absence or presence: left ventricular (LV) size and systolic function, right ventricular (RV) size and systolic function, and LV aneurysm. We included 725 patients with a mean age of 63.4 ± 12.3 years, 483 (67%) female. Abnormal electrocardiogram was observed in 81.5% of the patients. Left and right ventricular dysfunctions were found in 103 (14%) and 49 (7%) of the patients, respectively. Sensitivity, specificity, positive predictive value and negative predictive value were 84%, 94%, 70% and 97% for LV enlargement and 81%, 93%, 68% and 97% for LV systolic dysfunction, respectively, and 46%, 99%, 60% and 98% for RV dilatation, and 37%, 100%, 100% and 96% for RV dysfunction, respectively. Inter and intraobserver agreement were 61% and 87% for LV enlargement and 63% and 92% for LV dysfunction, respectively, and 50% and 49% for RV size and 46% and 79% for RV dysfunction, respectively. LV apical aneurysm was found in 45 patients (6.2%) with the lowest sensitivity of FoCUS study (11%; 95% CI 2-28%). FoCUS showed satisfactory indices of validity and reliability for assessment of cardiac chambers in patients with Chagas disease, except for apical aneurysm. This tool can identify heart disease with potential impact on patient management in the limited-resource setting.

845

HIGH BURDEN OF VACCINE PREVENTABLE DISEASES AS CAUSE OF DEATH: USING INNOVATIVE POST-MORTEM SAMPLING, THE ETHIOPIAN CASE

Hiwot Yizgaw[1], Mussie Berhane[1], Tseyon Tesfaye[1], Mersan Deresa[1], Mulu Berihun[1], Joseph Oundo[2], Nega Assefa[1], Lola Madrid[2]

[1]*Haramaya University, Harar, Ethiopia,* [2]*London School of Hygiene & Tropical Medicine, London, United Kingdom*

Only 43% of children aged 12-23 months in Ethiopia have received all basic vaccinations and ~20% have not received any vaccine. Pneumonia is a leading cause of death (CoD) and measles outbreaks are commonly reported in this country. Minimally invasive tissue sampling (MITS) is an innovative diagnostic tool, allowing accurate determination of infectious diseases as CoD in children, including vaccine-preventable diseases (VPDs). A surveillance of stillbirths and under five children (U5) started in a demographic surveillance (DSS) area in Eastern Ethiopia in February 2019 as part of Child Health and Mortality Prevention Network (CHAMPS). A death notification system was implemented to detect MITS-eligible cases (deaths occurring <24 hours earlier, belong to DSS and body available before burial). Conventional microbiology, multiplex PCR and histopathology were done on tissue samples. CoD was decided for each case by a panel of experts in different medical fields after reviewing clinical information, laboratory results and verbal autopsy. From 4th February 2019 to 3rd February 2020, 287 deaths were notified from inside the DSS. Deaths included 60 stillbirths (20.9%), 109 neonates (38.1%), 118 children aged 1-59 months (41.0%). Among 99 MITS eligible cases approached, 59 (59.6%) consented. CoD was given in 53 cases, and 13 (24.5%) were beyond neonatal period. In six of them (46.2%), a vaccine-preventable disease was identified as immediate CoD, including *Streptococcus pneumoniae (N=4*, one sepsis co-infected with *Neisseria meningitides* and three pneumonias, two with measles co-infections), *Neisseria meningitides* meningitis (N=1), and *vibrio cholerae* (N=1). Although MITS could only be collected on a small percentage of deaths, the process provided high quality data on CoD, being able to accurately detect infectious diseases as CoD. VPDs cause child deaths in a country where universal immunisation is offered for free in all regions. Given the unacceptably high childhood mortality rates in Ethiopia, greater access and wider use of immunizations are important to reduce child mortality.

846

OPEN SOURCE MONITORING FOR CLINICAL TRIALS IN CHALLENGING INFORMATION ENVIRONS

Rhys O'Neill

Novetta, McLean, VA, United States

In challenging environments with limited access to rapidly access information, accurate details concerning novel diseases and preventative measures to combat the growth of an outbreak often reach communities in an incomplete, piecemeal fashion. The absence of information often leaves media personnel or authoritative figures to fill those gaps and can lead to the dissemination of inaccurate descriptors or misinformation. Through Novetta's approach to tracking print and broadcast media (radio, television) in parallel to social media, we have supported communication arms of Ebola clinical trials in both West Africa and Central Africa in an attempt to identify where information gaps are prevalent and what communication systems are failing to reach vulnerable populations, particularly those outside of regions with accessible or adequate healthcare infrastructure. Discovering and defining damaging misinformation and disinformation in these environments allows Novetta to map geospatial relationships between the prevalence of this inaccurate content with the availability of appropriate communication channels to qualify target locations as "high risk." This method is intended to notify the ground communication teams and promotes targeted corrective messaging that limits the risk of community resistance to preventative measures. Novetta has expanded monitoring efforts to include the entire continent of Africa to support regional CDCs during the spread of COVID-19.

848

DELAYS IN HEPATITIS C FIBROSIS STAGING ON TREATMENT RETENTION

Austin T. Jones[1], Lisa Moreno-Walton[2], Torrence Tran[1], Christopher Briones[1], Rachael Stevens[1], Katharine Isaacson[1], Alexander Jafari[1], Mandy Majidian[1], Patricia Kissinger[1]

[1]*Tulane University, New Orleans, LA, United States,* [2]*Louisiana State University Health Sciences Center, New Orleans, LA, United States*

The WHO aims to treat 80% of hepatitis C virus (HCV) cases by 2030 by adopting a "Treat All" approach. Fibrosis staging is essential to determine choice and duration of HCV therapeutic regimen. The study objective was to evaluate time to fibrosis staging among those chronically infected with HCV and its association with achieving HCV cure. A retrospective cohort

study was conducted among chronic HCV-infected patients diagnosed in an emergency department in New Orleans, Louisiana from 2015 to 2017. Exposure was time from diagnosis of chronic HCV infection to fibrosis staging by transient elastography. Outcomes included treatment initiation, treatment completion, and sustained virologic response at 12-weeks (SVR-12). Multivariable linear regression was used to calculate risk differences. A total of 2,557 patients were found chronically HCV infected and 824 (34.2%) underwent fibrosis staging by transient elastography. Following diagnosis of chronic HCV infection, median time to fibrosis staging was 6.4 months (IQR 13.5). HCV treatment was initiated in 245 (29.7%) patients receiving fibrosis staging, a median of 13.5 months (IQR 19.1) following confirmation of chronic HCV infection. Each six-month delay in fibrosis staging decreased the likelihood of initiating treatment by 0.96% (p=0.45). Completion of HCV treatment occurred in 197 (23.9%) patients receiving fibrosis staging, a median of 16.4 months (IQR 16.8) following confirmation of chronic HCV infection. Each six-month delay in fibrosis staging decreased the likelihood of completing treatment by 0.80% (p=0.57). SVR-12 was achieved in 143 (17.4%) patients undergoing fibrosis staging, a median of 19.1 months (IQR 13.1) following confirmation of HCV chronic infection. Each six-month delay in fibrosis staging decreased the likelihood of SVR-12 by 4.51% (95% CI=1.07%-7.95%, p=0.01). Delay in fibrosis staging by transient elastography led to decreased HCV cure, although did not significantly affect treatment initiation or completion. Interventions should focus on decreasing delays in fibrosis staging in order to achieve the WHO "Treat All" goal.

848

DELAYS IN HEPATITIS C FIBROSIS STAGING ON TREATMENT RETENTION

Austin T. Jones[1], Lisa Moreno-Walton[2], Torrence Tran[1], Christopher Briones[1], Rachael Stevens[1], Katharine Isaacson[1], Alexander Jafari[1], Mandy Majidian[1], Patricia Kissinger[1]

[1]*Tulane University, New Orleans, LA, United States,* [2]*Louisiana State University Health Sciences Center, New Orleans, LA, United States*

The WHO aims to treat 80% of hepatitis C virus (HCV) cases by 2030 by adopting a "Treat All" approach. Fibrosis staging is essential to determine choice and duration of HCV therapeutic regimen. The study objective was to evaluate time to fibrosis staging among those chronically infected with HCV and its association with achieving HCV cure. A retrospective cohort study was conducted among chronic HCV-infected patients diagnosed in an emergency department in New Orleans, Louisiana from 2015 to 2017. Exposure was time from diagnosis of chronic HCV infection to fibrosis staging by transient elastography. Outcomes included treatment initiation, treatment completion, and sustained virologic response at 12-weeks (SVR-12). Multivariable linear regression was used to calculate risk differences. A total of 2,557 patients were found chronically HCV infected and 824 (34.2%) underwent fibrosis staging by transient elastography. Following diagnosis of chronic HCV infection, median time to fibrosis staging was 6.4 months (IQR 13.5). HCV treatment was initiated in 245 (29.7%) patients receiving fibrosis staging, a median of 13.5 months (IQR 19.1) following confirmation of chronic HCV infection. Each six-month delay in fibrosis staging decreased the likelihood of initiating treatment by 0.96% (p=0.45). Completion of HCV treatment occurred in 197 (23.9%) patients receiving fibrosis staging, a median of 16.4 months (IQR 16.8) following confirmation of chronic HCV infection. Each six-month delay in fibrosis staging decreased the likelihood of completing treatment by 0.80% (p=0.57). SVR-12 was achieved in 143 (17.4%) patients undergoing fibrosis staging, a median of 19.1 months (IQR 13.1) following confirmation of HCV chronic infection. Each six-month delay in fibrosis staging decreased the likelihood of SVR-12 by 4.51% (95% CI=1.07%-7.95%, p=0.01). Delay in fibrosis staging by transient elastography led to decreased HCV cure, although did not significantly affect treatment initiation or completion. Interventions should focus on decreasing delays in fibrosis staging in order to achieve the WHO "Treat All" goal.

849

MAMMALIAN TARGET OF RAPAMYCIN IN CHILDHOOD MALNUTRITION: QUESTING FOR CONNECTION

Parag Palit, Md Amran Gazi, Subhasish Das, Md Mehedi Hasan, Md Mehedi Hasan, Jafrin Ferdous, Sharika Nuzhat, Zannatun Noor, Mustafa Mahfuz, Rashidul Haque, Tahmeed Ahmed

icddr,b, Dhaka, Bangladesh

No attempt has been made to study the components of intracellular signaling pathways underlying cellular growth with regards to childhood malnutrition. Hence, we aimed to study the evident phenotype of childhood malnutrition by exploring possible implications of phosphorylated-Mammalian Target of Rapamycin Complex (p-mTORC), pivotal component in the mTOR pathway. Methodology: Venous blood was collected from 124 participants, aged between 8-18 months of either sex, equally distributed in each study group namely: stunted (LAZ ≤ -2), mildly stunted (-1 ≤ LAZ ≤ -2) and severely malnourished (WLZ ≤ -3 or having bilateral pedal edema) and healthy controls (LAZ, WAZ, WLZ ≥ -1). Peripheral blood mononuclear cells were isolated by density-gradient centrifugation and subsequently lysed for extraction of total intracellular protein using in-house cell lysis buffer. Levels of p-mTORC were assayed using commercial ELISA kits. Mann-Whitney U test was used to compare the optical densities (OD) for p-mTORC at 450 nm for stunted, mildly stunted and severely acute malnourished groups with that of the healthy controls. Bi-variate correlations between the OD for p-mTORC and different anthropometric outcomes by Spearman Rank correlation test. Results: OD for p-mTORC was significantly lower (p=0.01) in the severely malnourished group compared to healthy controls. No statistical significance was found between the OD for p-mTORC in the stunted group (p=0.118) and in the mildly stunted group (p=0.498) compared to that of the healthy controls, respectively. Significant weak positive correlations were observed between the OD for p-mTORC and anthropometric outcomes of LAZ (ρ=0.220, p=0.014), WLZ (ρ=0.265, p=0.003) and WAZ (ρ=0.311, p=0.000). Conclusion: To our knowledge, this is the first investigation that demonstrates potential implication of a key intracellular component of growth with childhood malnutrition. However, expression analyses of crucial signaling pathway genes are required to establish stronger links between the mTOR pathway and the phenotypic aberration of childhood malnutrition.

850

RISK FACTORS AND OUTCOMES ASSOCIATED WITH INCREASED MORTALITY DUE TO CHOLERA INFECTION IN PEDIATRIC POPULATIONS IN LMIC SETTING: A CASE FOR THE DOMINICAN REPUBLIC

Edgar Reynoso Vanderhorst[1], Miguel Delgadillo[2], Victoria Arís-Sanchez[2], Carla Mendoza-Valiente[2], Jose Moreira-Musa[2], Alejandro Blaubach[2], Kamila Guerrero-Richiez[2], Laura Manosalvas[1], Leandro Tapia[1], Robert Paulino-Ramirez[1]

[1]*Institute For Tropical Medicine & Global Health, Distrito Nacional, Dominican Republic,* [2]*School of Medicine, Universidad Iberoamericana (UNIBE), Santo Domingo, Dominican Republic*

Despite being often overlooked by developed countries, cholera infections remain a significant issue for LMIC. In many cholera-endemic countries, pediatric populations account for more than half of the incidence and deaths. Since its reintroduction in the Dominican Republic in 2010, increased hospitalizations by cholera infections present a high burden to healthcare services in the Dominican Republic. This study aims to quantify risk factors associated with mortality in cholera infected pediatric patients during the period 2012-2018 in the Dominican Republic. Cholera reports included in the Governmental Immunization Program and their demographic characteristics were extracted from the Ministry of Health Weekly Reports. A total of 1430 cases of pediatric cholera infections were reported during the studied period, with a mortality rate of 1.8% (n=26). Patients 0-9 years-old accounted for 61.1% (n=874) of the total reports. This age group was associated with an increased mortality of

4.9x (p=0.003). Hospitalizations accounted for 92.2% (n=1318) of the total reports and ambulatory care was associated with increased mortality by 3.6x (p=0.007). History of fever was observed in 18.2% (n=260) of reports and was associated with increased mortality by 3.4x (p=0.001). History of fever was associated with lower age, being most common in ages 0-9-year-old (OR: 1.5; p=0.02). An increased difference in mortality rate was not appreciated during outbreak settings (p=0.154). Evidence suggests an increased severity and mortality with younger age groups affected by cholera. Since ambulatory care was associated with increased mortality in pediatric populations, hospitalization guidelines should be revisited to attest for the difference in outcome. The presence of fever was associated with decreased age and increased mortality, making it an important indicator for clinical alarm. Recognizing that a younger age, history of fever, and ambulatory care contribute to increased mortality in pediatric patients, it is important to prioritize identification of these three factors in order to help prevent mortality and improve overall patient outcomes.

851

A RETROSPECTIVE REVIEW OF PATIENTS SEEN AT THE NATIONAL SCHOOL OF TROPICAL MEDICINE CLINIC AT BAYLOR COLLEGE OF MEDICINE IN HOUSTON, TEXAS FROM 2011-2020

Julika Kaplan, Fernando H. Centeno, Jill E. Weatherhead, Laila Woc-Colburn

Baylor College of Medicine, Houston, TX, United States

People living in Texas are vulnerable to neglected tropical diseases (NTDs), which cause extreme morbidity globally, due to the local climate and extreme poverty. Baylor College of Medicine's National School of Tropical Medicine founded a Tropical Medicine Clinic in Houston, Texas in 2011. The mission of this clinic is to prevent, diagnose, and treat NTDs. We conducted a retrospective chart review of all patients presenting to the Tropical Medicine Clinic within the Harris Health System, a healthcare safety net in Houston, between October 2011 and February 2020. We reviewed patients' electronic medical records to determine their demographic information, reasons for referral, and final diagnoses. 234 patients were seen at the clinic with 380 total encounters. Patients were referred for the following diagnoses: active tuberculosis (56; 24%) including pulmonary tuberculosis (30; 13%), tuberculosis lymphadenitis (17; 7%), and tuberculosis uveitis (9; 4%); neurocysticercosis (38; 16%); latent tuberculosis (24; 10%); Chagas disease (15; 6%); eosinophilia (7; 3%); histoplasmosis (7; 3%); coccidioidomycosis (7; 3%); and cases of schistosomiasis, echinococcosis, brucellosis, Mycobacterium avium complex infection, malaria, leishmaniasis, strongyloidiasis, chikungunya, leprosy, cryptococcosis, toxoplasmosis, and delusional parasitosis. Most patients were originally from Mexico (83; 35%) or Central America (41; 18%) including El Salvador, Honduras, Guatemala, and Nicaragua. However, many patients were born in the United States (27; 12%), some with recent foreign travel (9) but others without (13). Fewer patients were from East or West Africa, South America, Southeast Asia, South Asia, the Middle East, and the Caribbean. The Tropical Medicine Clinic in Houston has provided care to people from all over the world presenting with a wide variety of NTDs. Although many NTDs were diagnosed in recent immigrants, others were identified in patients who had lived in Houston for decades, supporting the idea that some of these infections are endemic to Texas. This highlights the importance of continuing to identify and treat NTDs in Houston.

853

CLINICAL SCORES AND PREDICTIVE MODEL TO DIFFERENTIATE BETWEEN *ANGIOSTRONGYLUS CANTONENSIS* AND *GNATHOSTOMA SPINIGERUM* IN EOSINOPHILIC MENINGITIS

Noppadol Aekphachaisawat[1], Kittisak Sawanyawisuth[2]

[1]*Silpakorn University, Bangkok, Thailand,* [2]*Khon Kaen University, Khon Kaen, Thailand*

Background: Angiostrongylus cantonensis and Gnathostoma spinigerum are the two most common causative agents for eosinophilic meningitis. Treatment is totally different for both parasites. Serological test is the most useful test to differentiate between these two parasites. Clinical factors may be helpful but limited. This study aimed to evaluate if clinical factors can be used to differentiate causes of eosinophilic meningitis between the two parasites. . Methods: We reviewed reported patients presented with eosinophils in cerebrospinal fluid from either *A. cantonensis* or *G. spinigerum* from literatures. The diagnosis must be confirmed either by serological tests or pathologically. Clinical factors between the two groups were analyzed by descriptive statistics and multivariate logistic regression analysis. Results: There are 155 definite cases of eosinophilic meningitis caused by these two parasites; 131 cases (84.5%) were in A. cantonensis group. There were 11 significant different factors between both groups by descriptive statistics such as incubation period, paresthesia, or xanthrochromic spinal fluid. Only two factors were independently associated with A. cantonensis group; radicular pain and motor weakness. The adjusted odds ratio (95% CI) of both factors were 0.015 (0.001, 0.204) and 0.020 (0.003, 0.147). Clinical score of over 58 were suggestive for gnathostomiasis with sensitivity of 87% and specificity of 96%. Conclusion: Presence of radicular pain and motor weakness were suggestive of Gnathostomiasis in eosinophilic meningitis patients.

854

POST-HOSPITAL SYNDROME: DOES HOSPITAL STAY CAUSE VULNERABILITY AFTER DISCHARGE IN INFANTS IN LOW-INCOME COUNTRIES?

Lison Rambliere[1], Perlinot Herindrainy[2], Agathe de Lauzanne[3], Laurence Borand[3], Elsa Kermorvant-Duchemin[4], Muriel Vray[5], Abdoulaye Seck[5], Awa Ndir[5], Michael Padget[6], Arnaud Tarantola[7], Touch Sok[8], Patrice Piola[2], Alexandra Kerléguer[3], Frédérique Randrianirina[2], Jean-Marc Collard[2], Didier Guillemot[1], Elisabeth Delarocque-Astagneau[9], Bich-Tram Huynh[1]

[1]*Institut Pasteur, INSERM, UVSQ, Paris, France,* [2]*Pasteur Institute in Madagascar, Antananarivo, Madagascar,* [3]*Pasteur Institute in Cambodia, Phnom Penh, Cambodia,* [4]*Hôpital universitaire Necker-Enfants malades, Paris, France,* [5]*Pasteur Institute in Senegal, Dakar, Senegal,* [6]*Pasteur Institut, INSERM, UVSQ, Paris, France,* [7]*Pasteur Institute in Cambodia, Paris, France,* [8]*CDC Department at Ministry of Health Cambodia, Phnom Penh, Cambodia,* [9]*INSERM, UVSQ, APHP, Paris, France*

After discharge from the hospital, patients face a transient period of generalized susceptibility to disease as well as an elevated risk for adverse events, including deaths and hospital readmissions that is called post hospital syndrome. This syndrome has been described recently and only in adults and in high-income countries, while no studies investigated the risk in children in low-income countries. Thus, the aims of this work are to estimate the burden of the post-hospital syndrome in terms of mortality and morbidity and identifying risk factors and the period of vulnerability after discharge among hospitalized infants in low-income countries. We used data from the BIRDY cohort, a community-based multi-centric paediatric study implemented in Madagascar, Cambodia and Senegal that followed 3,651 infants from birth to up to 24 months. Mortality, infectious events (diarrhea, respiratory infection, sepsis) were considered. In order to estimate the burden of post-hospital syndrome, hospitalized infants were compared with those not hospitalized with survival analysis after stratification matching using propensity score. Globally, 598 infants (16%) were hospitalized at least once, with the great majority during the two

first weeks of life in Madagascar and Senegal. The three quarters of deaths (54/72) occurred during the neonatal period of which half (25) occurred at home within one month after discharge. Compared to matched infants with health adverse events without a hospitalization, we showed that hospitalized infants were more at risk of death (Madagascar: p<0.01, Cambodia: p=0.03) and very severe infection (p<0.01, p=0.03), diarrhea (Cambodia: p>0.01) and bronchiolitis (p=0.05) within the six months after discharge. Our results showed that hospitalization during the first weeks of life is clearly at high risk of death after discharge. Hospitalized infants were also particularly at higher risk of infections several months after discharge. These children represent an accessible high-risk population in which targeted interventions to prevent morbidity and mortality are clearly needed.

855

PRELIMINARY FINDINGS FROM THE FIEBRE STUDY IN MOZAMBIQUE: CLINICAL FINDINGS AND RESULTS OF POINT-OF-CARE TESTS AND MICROBIOLOGY STUDIES

Marta Valente[1], Sara Ajanovic[1], Justina Bramugy[2], Pio Vitorino[2], Sham Lal[3], David Mabey[3], Quique Bassat[1], Heidi Hopkins[3]

[1]*Isglobal-CISM, Barcelona, Spain,* [2]*Centro de Investigaçao em Saúde de Manhiça, Manhiça, Mozambique,* [3]*London School of Hygiene & Tropical Medicine, London, United Kingdom*

Many febrile illnesses present with non-specific symptoms and signs, and this often results in them being misdiagnosed and inappropriately treated and may encourage antimicrobial resistance development. Current WHO algorithms for primary care in low and middle-income countries do not provide comprehensive guidance for management of non-malarial fevers. Mozambique, with a high prevalence of *human immunodeficiency virus* (HIV), faces even more challenges due to the potential variety of etiologies and opportunistic infections presenting. We present preliminary findings from the Mozambique site of the multi-centre observational study "FIEBRE: Febrile illness evaluation in a broad range of endemicities". The primary objective of the study is to describe the infectious causes of fever, and antimicrobial susceptibility of identified bacterial species, among inpatients and outpatients aged ≥2 months. Recruitment in Mozambique began in November 2018 and is ongoing to meet the target sample size of 2400 patients and 600 controls. Point-of-care (POC) tests including malaria rapid diagnostic test and microscopy, HIV rapid test, and urine dipstick, as well as blood and urine culture, are performed for all patients; mycobacterial blood culture, cryptococcal antigen serum rapid test, and urine lipoarabinomannan rapid test are performed for patient subgroups. Samples are archived for future pathogen-specific reference laboratory testing. To date 1556 patients have been enrolled (65% of the target). Preliminary data (as of October 2020) on POC and microbiology test results, along with relevant clinical data, from the Mozambique study population will be presented, comparing findings stratified by patient age, HIV status, and other clinical and demographic features. Once complete, FIEBRE study data are expected to contribute to updates for the current recommendations on the clinical management and prevention of febrile illnesses in low and middle-income countries, and to support estimates of the incidence of fever-causing agents.

856

CUTANEOUS LEISHMANIASIS AND HEALTH-RELATED QUALITY OF LIFE IN RETURNING TRAVELERS TO THE UNITED KINGDOM

Emilia Peleva, Stephen L. Walker

University College London Hospitals NHS Foundation Trust, London, United Kingdom

Leishmaniasis is a vector-borne parasitic neglected tropical disease. Cutaneous leishmaniasis (CL) is associated with reduced health-related quality of life (HRQoL) and psychological distress in endemic countries. There is little information on CLs impact on HRQoL in returning travelers. We conducted a retrospective cohort study of individuals with CL treated at the Hospital for Tropical Diseases between 1st March 2018 and 1st March 2020. Patient demographics, disease characteristics and Dermatology Life Quality Index (DLQI) scores were summarized. 50 patients with laboratory confirmed CL were included. 70% (n=35) were male and age ranged between 2 to 76 years (mean 39.1, SD 19.0). One patient was immunosuppressed and no patients had HIV. Most patients (n=36, 72%) had a single lesion, 6 patients (18%) had two lesions, and 8 patients (16%) had three or more lesions. Lesions were distributed on the lower limb (n=19, 38%), head and neck (n=18, 36%), upper limb (n=17, 34%), and other sites (n=5). The size of skin lesions ranged from 0.5-10cm. The causative species identified using PCR were *L. Viannia* complex in 27 patients (54%), *L. donovani* complex in 9 patients (18%), *L. major* in 8 patients (16%), *L. mexicana* in 2 patients, *L. tropica* in 2 patients, and unknown species in 2 patients. The likely regions of acquisition were the Americas (n=29, 58%), Middle East (n=8), Europe (n=8), Africa (n=4) and South Asia (n=1). DLQI data were available for 40 patients (80%). The mean total DLQI score was 7.4 (SD 4.8, range 0-20), with a very large or moderate effect in 25 (50%) patients. The highest effect was observed on the physical symptoms and feelings, leisure, and work/school domains. The lowest effect was in the daily activities and relationships domains. Larger lesions were associated with reduced HRQoL (p <0.05). No significant difference was observed with respect to age, gender, or number of lesions present. CL was found to be associated with a significant impact on the HRQoL in returning travelers. The findings in travelers are similar to those of individuals living in endemic countries such as Iran, Turkey and Brazil.

857

ESTABLISHING QUALITY CONTROL MEASURES TO MAXIMIZE DATA QUALITY OF A NEW ONCHOCERCIASIS ELISA KIT

Holly M. Chastain[1], E. Scott Elder[1], Henry M. Kanyi[2], Sammy M. Njenga[2], Joseph Kamgno[3], Adjami Aime Gilles[4], Anthony Luyai[4], Jeffrey W. Priest[1], W. Evan Secor[1], Kimberly Won[1]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Kenya Medical Research Institute, Nairobi, Kenya,* [3]*Center for Research on Filariasis and other Tropical Diseases, Yoaunde, Cameroon,* [4]*Expanded Special Project for Elimination of NTDs Laboratory, Ouagadougou, Burkina Faso*

High quality diagnostic tools are critical for making appropriate intervention decisions in neglected tropical disease programs. The Onchocerciasis Ov16 IgG4 ELISA kit (Standard Diagnostics, Seoul, South Korea) has been proposed for use in onchocerciasis elimination programs. For this test, we sought to establish quality control measures using control sample data generated in Tanzania, Burkina Faso and Kenya. We applied those parameters to data generated in three laboratories in Cameroon, Kenya and the United States. Each kit contained 3 manufacturer provided controls and a calibrator for plate normalization. We considered test sample values >0.5 after normalization to be positive. Acceptable plates must have had normalized positive control values >0.5 and normalized negative control values <0.5. We defined an acceptable range for calibrator values as +/- 2 standard deviations (SD) of values from the first 100 plates from each laboratory. Any plate with a calibrator value outside the established range was rejected. We established a range for normalized positive controls using the same process; plates with values outside this range were rejected. We used 3 concentrations (2 ng/ml, 1 ng/ml, 0.5 ng/ml) of a humanized monoclonal antibody (mAb) (BioRad) as external controls on each plate. We used a Westgard based process for plate acceptance criteria for the mAb. A range was established for each concentration of the mAb after the first 20 plates; 2 of the 3 mAb results had to be within 2 SD of the mean. Each acceptable value was then progressively added to the mean calculations. We rejected duplicate calibrator or positive control values with coefficient of variation >20%, and individual sample results if duplicate results were discordant. After applying these standards, Kenya, Cameroon, and USA laboratories had sample repeat rates of 7.8%, 5.5% and 7.0%, respectively. By blending methods of quality measures, we effectively established a set of parameters to monitor Ov16 ELISA data quality across laboratories.

858

THE PREVALENCE OF DREPANOCYTARY CHILDREN IN THE CITY PROVINCE OF KINSHASA / DRC

Basudikila Mvumbi, M. G Bile

Ministry of Health, Kinshasa, Democratic Republic of the Congo

Sickle cell disease is a constitutional hemoglobinopathy, with autosomal transmission the most widespread in the world with 400,000 new births of sickle cell patients. 70% of cases are in sub-Saharan Africa. The DRC is the 2nd country in Africa after Nigeria with 2 to 3% of SS births, 50-75% of which die before the age of 5 and 25 to 30% of heterozygous births. Aim: Determine the extent of the disease in the city of Kinshasa province 2011-2018.Describe the profile of parents with sickle cell children and those with children without sickle cell in the city of Kinshasa province. Epidemiological data were collected in 25 health zones by document review and interview with providers; survey data was collected in households from 504 parents (252 with sickle cell children and 252 others with non-sickle cell children) by maintenance. the data was organized on Excel and processed on spss and spinx v. Sickle cell disease remains a major problem in the city of Kinshasa province with a high lethality of 61%, a high mortality of children <5 years and pregnant women with sickle cell disease 50%. In conclusion, the low level of education, ignorance of the disease and electrophoretic status before marriage as well as clan marriage are the factors associated with the high prevalence of the disease (sickle cell anemia) in the city of Kinshasa province

859

FACTORS ASSOCIATED WITH THE OCCURRENCE OF EPILEPSY IN SIX HEALTH DISTRICTS IN MALI IN 2019: CASE-CONTROL STUDY

Housseini Dolo[1], Fatoumata Nènè Konipo[2], Yaya Ibrahim Coulibaly[3], Moussa Sangare[1], Mariam Daou[4], Hassane Diallo[4], Samba Oumar Djimdé[4], Sekouba Goita[4], Harouna Kone[2], Fousseini Diadouda Coulibaly[2], Mohamed Fako Keita[2], Adama Karembe[2], Orsot Kissy Elvira[2], Abdou Koita[4], Birama Sangare[4], Tenimba Bagayoko[4], Aly Badhara Dem[4], Zoumana Fomba[4], Mamadou Gari[4], Chimène Essi Kotchene[4], Youssoufa Mamadou Maiga[5], Seydou Doumbia[2]

[1]*Faculty of Medicine and Odonto Stomatology / Department of Teaching and Research in Public Health and Specialties / Lymphatic filariasis research Unit, International Center of Excellence in Research, Bamako, Mali,* [2]*Faculty of Medicine and Odonto Stomatology / Lymphatic filariasis research Unit, International Center of Excellence in Research, Bamako, Mali,* [3]*Faculty of Medicine and Odonto Stomatology / Department of Teaching and Research in Public Health and Specialties / Lymphatic filariasis research Unit, International Center of Excellence in Research / Dermatology Hospital of Bamako, Bamako, Mali,* [4]*University Teaching Hospital, Gabriel Touré, Bamako, Mali,* [5]*Faculty of Medicine and Odonto Stomatology / University Teaching Hospital, Gabriel Touré, Bamako, Mali*

Epilepsy is a chronic, cosmopolitan neurological condition with medical, psychological, economic and social repercussions. Stigma and discrimination prevent people from seeking care. The objective of this study was to identify factors associated with the occurrence of epilepsy in six health districts (HDs) of Mali selected in 3 different eco-climatic areas (Kayes, Kéniéba, Kolokani, Sikasso, Kadiolo and Tominian). A case-control study was conducted from June to December 2019 in the 6 HD of Mali. During the data collection, each confirmed case of epilepsy was matched with two controls from the same village, by gender and age group. A questionnaire assessing socio-demographic characteristics as well as family and medical history was administered to each participant. A total of 3,846 participants including 1,645 cases and 2,201 controls were investigated. The assessment of factors associated with the occurrence of epilepsy led to the understanding that the risk of having epilepsy did not differ significantly between the different study districts. Duration and type of delivery were associated with epilepsy with respectively OR=3,29, 95% CI [2,22-4,87] and OR=4,54, 95% CI [2,89-7,12]. Prematurity was also observed to be a contributing factor in the occurrence of epilepsy, OR=2.22, 95% CI [1.44-3.33]. We also observed that consanguinity was significantly associated to the occurrence of epilepsy OR=1.21, 95% CI [1.05-1.41]. In terms of infectious diseases, a history of meningitis was found as a significant risk factor for the occurrence of epilepsy in the study communities, OR=2.56, 95% CI [1.63-4.0]. More importantly, a history of cerebral malaria was found as a major risk factor in the occurrence of epilepsy OR=12.04, 95% CI [10-16.66]. In rural areas of Mali, a history of cerebral malaria or meningitis, prematurity and long and difficult childbirth were factors associated with the occurrence of epilepsy. These findings support the need for improving malaria and meningitis control, reproductive and maternal health deficiency in such rural tropical areas.

860

TESTING DIFFERENT WHO HEAD CIRCUMFERENCE Z-SCORE CUTOFFS FOR ASSOCIATION WITH NEURODEVELOPMENTAL OUTCOMES IN INFANTS AND YOUNG CHILDREN IN RURAL GUATEMALA

Molly M. Lamb[1], Amy K. Connery[2], Alison M. Colbert[2], Desiree Bauer[1], Daniel Olson[2], Alejandra Paniagua-Avila[3], Paola Arroyave[3], Sara Hernandez[3], Maria A. Martinez[3], Andrea Holliday[4], Hana M. El Sahly[5], Flor M. Munoz[5], Edwin J. Asturias[2]

[1]*University of Colorado School of Public Health, Aurora, CO, United States,* [2]*Childrens Hospital Colorado, Aurora, CO, United States,* [3]*Fundacion para la salud integral de los guatemaltecos, Coatepeque, Guatemala,* [4]*EMMES Corporation, Rockville, MD, United States,* [5]*Baylor College of Medicine, Houston, TX, United States*

There is no universally accepted metric to determine clinically meaningful microcephaly in Low- and Middle-Income country (LMIC) populations, as the association between head circumference (HC) in early life and neurodevelopment (ND) has been understudied in these populations. We tested the different WHO z-score cutoffs used to define microcephaly for association with ND in infants and young children in Guatemala. HC of 424 infants (age 0-16 months) and 100 young children (age 21-36 months) was measured during a study of postnatal Zika infection in rural Guatemala, and z-scores were calculated using WHO growth charts. The Mullen Scale of Early Learning (MSEL) was administered to measure neurodevelopmental skills. We conducted generalized linear regression analyses to identify which published WHO microcephaly definitions (WHO z-score cutoffs = -1, -1.28, -1.5, -2) were most associated with worse ND. No study subjects contracted Zika during the study period. In infants, the HC z-score cutoffs were not associated with lower MSEL standard scores. In young children, there was a significant association between having a HC z-score below a cutoff and lower MSEL standard scores (Relative Risk (RR) ~ 0.89, 95% Confidence Interval ~ 0.83 - 0.97). A trend for increasing strength of this association with more stringent WHO z-score cutoff was observed (WHO z-score cutoff = -1: RR = 0.93; Z-score cutoff = -1.28: RR = 0.91; Z-score cutoff = -1.5: RR = 0.89; Z-score cutoff = -2: RR = 0.85). Small head size was associated with poor neurodevelopment in young children, but not in infants. More stringent WHO z-score cutoffs to define microcephaly had increasingly strong association with poor ND. These results suggest that a variety of WHO z-score cutoffs can be used effectively to detect microcephaly, and that measuring HC in young children could be helpful to identify those in need of neurodevelopmental testing and intervention.

861

MARKERS OF SERIOUS BACTERIAL ILLNESS IN CHILDREN IN A MALARIA ENDEMIC SETTING

Benetta Andrews[1], Jonggyu Baek[2], Patricia A. McQuilkin[2]

[1]*Liberian College of Physicians and Surgeons, Monrovia, Liberia,* [2]*University of Massachusetts, Worcester, MA, United States*

Background: In malaria endemic countries, it is often difficult to distinguish between fever caused by malaria and that caused by serious bacterial illness (SBI). Studies performed in non-malaria endemic countries have

suggested that biomarkers such as white blood cell count (WBC), absolute neutrophil count (ANC) and C-Reactive Protein (CRP) can be helpful in identifying children with SBI. Such tools would be extremely useful in malaria endemic settings. We undertook a prospective hospital-based study in Liberia, a West African country with a high malaria burden, to determine if biomarkers such as WBC, CRP and ANC could help to distinguish between malaria and SBI in children. Methods: The study site, JFK Medical Center, is a tertiary care center located in Monrovia. Children aged 6 months - 5 years presenting with fever within 24 hours were invited to participate. Demographic and clinical information was collected. All patients had a CBC , blood culture , malaria smear and CRP level performed. Other labs tests and studies were performed to identify children with SBI. Descriptive statistics, and chi square analyses were performed to determine measures of association. Ethical approval was obtained by the JFKMC IRB. Results Of a total of 244 patients, 220 were part of the analysis, with the mean (SD) age 22.9 (14.3) months. We found that 18.2% of children presenting with fever had malaria, 10.5%, had an SBI and 1.8% were co-infected. For 65.4% of children, no source was found. We looked at the following biomarkers cutoffs in all patients : CRP > 20 mg/dl, ANC>5000 and WBC > 15,000. Of these markers, CRP > 20 was significantly associated with infection with malaria adjusting for age (P<0.0016). ANC and WBC were not significantly associated with either type of infection. Conclusions In this population of pediatric patients, CRP was highly predictive of malarial illness and not a useful tool in identifying patients with SBI; WBC and ANC were not associated with either type of infection. Limitations of our study included a small sample size, which may not be generalizable. Future studies will involve elucidating the viral etiologies of fever in this population.

862

DEVELOPING CLINICAL RESEARCH CAPACITY IN LIBERIA

Patricia McQuilkin[1], Troy Moon[2], Moses Badio[3], Jonggyu Baek[1], Nicola Bulled[1], Steven Hatch[1], Stuart Levitz[1], Ann Moormann[1], Soka Moses[3], Sanjay Ram[1], Richard Ssekitoleko[1]

[1]*University of Massachusetts, Worcester, MA, United States,* [2]*Vanderbilt University Medical School, Nashville, TN, United States,* [3]*PREVAIL, Monrovia, Liberia*

Since 2018, a consortium consisting of the Liberian College of Physicians and Surgeons (LCPS) and U.S. Academic partners has been working to develop clinical research capacity in Liberia. The objectives of this program are to 1) foster an interest in research; 2) introduce basic research concepts; 3) support the development of pilot projects and 4) identify Liberian leaders who can carry research training forward. Methods The research training program was implemented in November 2019 at JFK Medical Center, the national teaching hospital. A needs assessment was conducted to design programming and identify barriers to performing research in this setting. We developed a training program consisting of four, one-week workshops, which were implemented over 12 months. Workshops were co-taught by US and Liberian faculty. Evaluations were performed with trainees before and after the workshop series. Three key barriers to performing research were identified: 1) few research mentors 2) lack of funding and 3) unreliable internet. To overcome these barriers, we installed internet to allow for better communication and access to the literature. We also awarded pilot funding on a competitive basis. Ten proposals were submitted, with seven approved for funding. Over 95 clinical trainees attended at least one workshop, with 23 attending the entire series, and an average attendance of over 50 trainees across specialty areas. Trainee reports of skills learned included: 1) identifying a research project and 2) performing a literature search. Identified areas of continued need included ongoing, mentored research training and specific skills in data analysis and grant writing. Conclusions: Based on feedback, a more specific, deliverable driven training program was developed and is currently being implemented. The second cohort will be smaller (20 trainees) and training will consist of hands on skill sessions and assigned, mentored research projects. This initial program fulfilled our objectives 1-3. We continue to strive to fulfill our 4th objective: to identify Liberian research leaders who will be trained to continue the training program

863

VALIDATION OF A LOW-COST MOBILE RETINAL SCANNER PROTOTYPE AS A SCREENING TOOL FOR RETINAL DISEASES IN MOZAMBIQUE

María Postigo[1], Laura Beltran-Agullo[2], Olivia Pujol[2], Rubao Bila[3], Jaime García[4], **Rosauro Varo**[5], Virginia García[2], Lucía Sallé[6], Adriana Mousa[1], Andrés Santos[6], María J. Ledesma-Carbayo[6], Quique Bassat[7], Miguel Luengo-Oroz[4]

[1]*SpotLab, SpotLab S.L, Madrid, Spain,* [2]*Institut Català de Retina, Barcelona, Spain,* [3]*Centro de Investigação em Saúde de Manhiça (CISM), Manhiça, Mozambique,* [4]*SpotLab, SpotLab S.L., Madrid, Spain,* [5]*Barcelona Institute for Global Health, Barceona, Spain,* [6]*Biomedical Image Technologies Group, DIE, ETSI Telecomunicación, Universidad Politécnica de Madrid & CIBER-BBN, Madrid, Spain,* [7]*Barcelona Institute for Global Health, Barcelona, Spain*

In developing countries, there are only a few studies on the prevalence of retinal disease, and retinal symptomatology in specific diseases has not yet been characterized (e. g: cerebral malaria, HIV, hypertension, diabetes). Easier access to retinal information would allow differential diagnosis and promote strategies to improve eye health, which are currently very scarce. This is a pilot study aims to evaluate the functionality and usability of a tele-retinography system for the detection of retinal pathology, based on a low-cost portable retinal scanner, manufactured with 3D printing and controlled by a mobile phone with an application designed ad-hoc. The study was conducted at the Manhiça Rural Hospital in southern Mozambique. General practitioners, with no specific knowledge of ophthalmology or previous use of retinography, performed digital retinographies on 101 hospitalised patients (202 eyes), for whom there was no previous ophtalmological information. The retinographies were acquired in video format. Through the application they were later uploaded to a web platform, and were reviewed by two ophthalmologists in Barcelona, analyzing the image quality, the presence of retinal lesions and providing a diagnosis. The videos had an average duration of 17. 4 seconds and a quality of 1. 52 (1-Good, 2-Soft, 3-Bad). In total, 16 videos (7. 9%) were of insufficient quality to make a diagnosis. Typically pathological lesions were observed in 15 patients out of 101, requiring further testing for confirmation. The tool used is easy to use by healthcare personnel without specialised ophthalmological knowledge and could be used for screening and initial diagnosis of retinal pathology.

864

COMPARATIVE ANALYSIS OF SEPSIS TREATMENT IN AUSTERE ENVIRONMENTS

Gary Fogel[1], Derren Barken[1], Deborah Striegel[2], Paul Blair[2], Misato Miyamasu[2], Josh G. Chenoweth[2], Subramaniam Krishnan[2], Joost Brandsma[2], Kevin L. Schully[3], Te Vantha[4], Marvin J. Sklar[5], Anne Fox[6], Andrew Letizia[7], George Oduro[8], Charmagne Beckett[7], Benjamin Espinosa[3], Danielle V. Clark[2]

[1]*Natural Selection, Inc., San Diego, CA, United States,* [2]*Austere Environments Consortium for Enhanced Sepsis Outcomes, Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD, United States,* [3]*Austere Environments Consortium for Enhanced Sepsis Outcomes, Biological Defence Research Directorate, Naval Medical Research Center-Frederick, Ft. Detrick, MD, United States,* [4]*Takeo Provincial Referral Hospital, Takeo, Cambodia,* [5]*Naval Medical Research Center - Asia, Singapore, Singapore,* [6]*Naval Medical Research Unit-3 Ghana Detachment, Accra, Ghana,* [7]*Infectious Diseases Directorate, Naval Medical Research Center, Silver Spring, MD, United States,* [8]*Komfo Anokye Teaching Hospital, Kumasi, Ghana*

Sepsis is a systemic inflammatory syndrome that can occur in response to an infection. Some patients may progress to severe sepsis or septic shock, leading to life-threatening decreases in blood pressure and organ failure. Military personnel are at a particular risk of sepsis when deployed to austere regions with limited resources. The Austere environments Consortium for Enhanced Sepsis Outcomes (ACESO) aims to improve

survival for sepsis patients in resource-limited settings through early recognition, diagnosis and evidence-based clinical management. Effective utilization of available sepsis treatments in low resource settings remains a major clinical challenge. Here, we analyze detailed longitudinal treatment data for two sepsis cohorts Cambodia (*n*=177) and Ghana (*n*=160) with similar per capita incomes, age distributions and dissimilar gender distribution (31% vs 51% female, respectively). The two cohorts had somewhat similar severity at hospitalization as measured by qSOFA (with Ghana higher) but vastly different clinical outcomes: Cambodia mortality at day 28 (d28) was 11.9% vs. Ghana 31.9% (p<0.001). The overall mean number of medications per subject was significantly higher for Cambodia vs. Ghana (p<0.001). Notably, this difference was not primarily due to antibiotic usage. Medication usage was compared using ATC classification codes. Statistically significant differences (p<0.05 FDR) in usage between the two sites were observed at ATC Level 1 (5 codes), ATC Level 2 (17 codes), ATC Level 3 (21 codes), and at the medication level (35 medications). Additionally, there were significant differences in pre- vs. post-hospitalization for first utilization of a medication, with Ghana having considerably more pre-hospitalization first utilization relative to Cambodia. Conversely, Cambodia had more frequent treatment adjustments than Ghana. Survival as measured by d28 and Kaplan-Meier plots was highly stratified by qSOFA in Ghana and less so in Cambodia due to lower overall mortality. The comparison of sepsis treatment by cohort provides insight towards clinical outcomes including mortality rates.

865

USE OF TAQMAN ARRAY CARD TECHNOLOGY IN DETERMINATION OF CAUSE OF DEATH FOR STILLBIRTHS AND UNDER-FIVE DEATHS IN EASTERN ETHIOPIA

Mussie Brhane[1], Mersan Deresa[1], Mulu Berihun[1], Dadi Marami[1], Zelalem Teklemariam[1], Fami Ahmed[1], Nardos Assegid[1], Lola Madrid[2], Joseph Oundo[2], Nega Assefa[1]

[1]*College of Health and Medical Science, Haramaya University, Harar, Ethiopia,* [2]*London School of Hygiene & Tropical Medicine, London, United Kingdom*

Child Health and Mortality Prevention Surveillance (CHAMPS) network has implemented custom multi-pathogen TaqMan Array Card (TAC) technology as a molecular testing method for detection of infectious agents contributing to stillbirths and under-five child mortality. A standardized process, known as Determination of Cause of Death (DeCoDe), was performed by a panel of experts to thoroughly analyze and review compiled data, including TAC results, to assign underlying and immediate causes for each under-five child death and stillbirth. We analyzed use of TAC technology in DeCoDe in the CHAMPS-Ethiopia site. Four custom multi-pathogen panels consisting of multiplex real-time PCR assays in TAC microfluidic system were used to test postmortem specimens (lung tissue, respiratory (nasopharyngeal/oropharyngeal) swab, rectal swab, whole blood, and CSF) collected from the deceased using a minimally invasive tissue sampling procedure. 53of 59 (89.8%) cases recruited from February 2019 to February 2020 were reviewed and assigned a final cause of death. Excluding stillbirths, 22 of 28 (78.6%) cases were infection-related deaths and TAC results were used to determine cause of death. A TAC results were the primary source of data considered by the DeCoDe expert panel in 12 of 13 (92.3%) of cases. Similarly, causes of death in 10 of 15 (66.7 %) neonatal in children beyond the neonatal periodcases were also determined based on TAC results. In addition, among the pathogens detected, 33 were determined as definitive cause of death consisting of bacteria (87.9%), viruses (9.1%) and parasites (3%). K. pneumoniae, S. pneumoniae, E. coli, and Salmonella species accounted for cause of death in more than two thirds (63.6%) of cases, with the remainder of bacterial detections being N. meningitidies, H. influenzae, U. urealyticum/parvum, Group A Streptococcus, Group B Streptococcus, V. cholerae, and P.aeruginosa. In conclusion, TAC was found to be valuable in determining cause of death in under-five children. We emphasize use of TAC as a multi-pathogen detection platform for disease surveillance, outbreak investigation and severe case diagnostics.

866

COMPARING PERCEIVED AND ACTUAL RISK OF TRAVEL-RELATED CONDITIONS AMONGST PAEDIATRIC AND ADULT TRAVELLERS AT A TERTIARY CARE CENTRE: A CROSS-SECTIONAL STUDY

Pierre-Philippe Piché-Renaud[1], Jenny Hoang Nguyen[1], Lisa G. Pell[1], Xiaowei Ma[1], Nadia Alattas[1], Sarah Khan[2], Kevin Schwartz[3], Nadia Akseer[1], Ray Lam[1], Debra Louch[1], Michelle Science[1], Shaun K. Morris[1]

[1]*Hospital for Sick Children, Toronto, ON, Canada,* [2]*McMaster Faculty of Health Sciences, Toronto, ON, Canada,* [3]*Public Health Ontario, Toronto, ON, Canada*

With the rise in global migration and travel, preventing morbidity from infections and other causes related to travel is increasingly important. Traveler self-risk perception for travel acquired infections and conditions is an important contributor to decisions, behaviours, and activities during travel. It is unknown how accurately travelers assess self-risk and their children's risk. To assess differences between perceived and actual risk of travel-related conditions in children and adults travelling internationally. Children <18 years and adults were recruited at the Family Travel Clinic at the Hospital for Sick Children between October 2014 and July 2015. A 7-point Likert scale questionnaire assessing risk perception of 32 travel-related conditions was administered. Most conditions were categorized under vector-borne diseases, vaccine-preventable diseases, food and water borne diseases and sexually transmitted infections. Composite scores for each of these categories were created using means. Two certified travel medicine experts reviewed each patient's chart, planned trip itinerary and activities and assessed an expert risk score. Perceived and expert risk scores were analyzed using paired T-tests. In total, 98 children (self-reported, n=9; adult-reported, n=89), and 110 adults were recruited. Travel-related risk for children (self-perceived and adult-perceived) was significantly underestimated compared to experts for 25 of the 32 assessed conditions. Adults also significantly underestimated their own travel-related risk for 26 of the 32 studied conditions, including the same conditions as children, as well as fever. Travel-related risk was not over-estimated for any condition by either children or adults. In conclusion, adults underestimated their children's and their own risk for most travel-related conditions. Further studies are needed to assess the impact of risk perception on travel-related behaviours and compliance to pre-travel advice, which will inform future approaches to mitigating risk and optimizing healthy travel for children and their families.

867

ADVERSE BIRTH OUTCOMES AND FACTORS ASSOCIATED AMONG LIVE BIRTHS AT SAINT PAUL'S HOSPITAL MILLENNIUM MEDICAL COLLEGE

Delayehu Bekele[1], Kimi Van Wickle[2], Mahlet Abayneh[1], Tsegaye Bezabih[1], Tsedeke Wolde[1], Semaria Solomon[1], Tefera Biteye[1], Yahya Mohammed[1], Oludare A. Odumade[3], Gesit Metaferia[1], Grace J. Chan[2]

[1]*St. Paul's Hospital Millenium Medical College, Addis Ababa, Ethiopia,* [2]*Harvard T.H. Chan School of Public Health, Boston, MA, United States,* [3]*Boston Children's Hospital, Boston, MA, United States*

Increasing evidence suggests that infants born with adverse birth outcomes may have various long-term health challenges. The aim of this study is to identify infant and maternal characteristics associated with adverse birth outcomes at Saint Paul's Hospital Medical College (SPHMMC) in Addis Ababa, Ethiopia. As part of a larger multi-country study on the Burden of Antibiotic Resistance in Neonates from Developing Societies (BARNARDS), we conducted a prospective cohort study at SPHMMC in Addis Ababa following 4,583 mother-newborn inborn pairs between March 2017 and February 2018. Newborns were followed over the first 7days of life. The primary outcome measure was adverse birth outcome, which included preterm birth, low birth weight, and early neonatal death. Preterm delivery was defined as live-singleton births that occurred after

28 weeks and before 37 gestational weeks. We defined low birthweight as birthweight less than 2500g. Early neonatal death was defined in the first 7 days of life. Bivariate and multivariable logistic regression models were conducted to assess the effect of maternal and infant characteristics on any adverse birth outcome from birth to 7 days of life. We repeated the analyses to examine associations on specific outcomes: preterm births, low birthweight, and early neonatal deaths. Of the 4,583 mother-newborn pairs, 1,262 (22.00%) had an adverse birth outcome. At birth, 676 (11.79%) newborns were preterm and 1075 (18.74%) were low birthweight. Within the first 7 days of life, 113 (1.97%) newborns died. After adjusting for potential maternal and infant confounders, increased risk of any adverse outcome was significantly associated with chronic hypertension (adjusted odds ratio [AOR] = 2.0, [1.4;2.6], p<0.01), history of vaginal bleeding (AOR=1.7, [1.0;2.8], p<0.01) and history of preterm births (AOR=4.6,[1.1;18.1], p<0.04). Chronic hypertension was a significant risk factor for both preterm (AOR=3.9,[2.8.9;5.4], P<0.01) and low birth weight babies (AOR=2.5,[1.9;3.4], P<0.01). Our analyses has identified key factors associated with adverse outcomes among live births in the Ethiopian setting for further study.

868

HUMAN INTESTINAL PARASITES ASSOCIATED WITH EGGPLANT (*SOLANUM AETHIOPICUM*) SOLD IN OGBARU LOCAL GOVERNMENT AREA ANAMBRA STATE NIGERIA

Nkeiruka Marykate Orji[1], Evelyn Nkechi Ezekwueme[1], Emmanuel Chidera Orji[2]

[1]*Chukwuemeka Odumegwu Ojukwu University, Uli, Anambra State, Nigeria,* [2]*Dezan Medical Diagnostic and Research Laboratory, Owerri, Nigeria*

The study was carried out to determine Human Intestinal parasites associated with *Solanum aethiopicum* in Ogbaru Local Government Area of Anambra State, Nigeria. Four Towns were involved in the study namely, Okpoko, Odekpe, Atani and Ochuche all in Ogbaru Local Government Area. A total of 800 samples were collected,200 batch of vegetables (100 batch of leaves and fruits respectively from each town). The prevalence of the intestinal parasites on the leaves and fruits of *S, aethiopicum* was examined using wet preparation methods(normal saline/iodine preparations) concentration methods (saturated sodium chloride flotation and zinc sulphate flotation methods respectively). Equally modified Ziehl Neelson staining method was employed for the identification of oocysts. Results obtained showed that of the 800 vegetables (Leaves and fruits) of *S, aethiopicum* examined, larvae of *Strongyloides stercoralis* had the highest prevalence rate (21.85%) followed by ova of *Ascaris lumbricoides* with prevalence rate of 21.64%,*G.lamblia* (18.07%),cyst of *E. histolytica* (17.86%),ova of hookworm (14.08%) while oocyst of *Cryptosporidium parvum* had the least prevalence rate of 6.51%. Equally Okpoko town has the highest number of intestinal parasites isolated from leaves/fruits of *S. aethiopicum* with a contamination rate of 27.52% while Odekpe had the least with a contamination rates of 22.48%. The findings of this study provide evidence that there is a potentially high risk of acquiring parasitic infections from the consumption of raw vegetables. Effort should be made by the relevant bodies to reduce the rate of parasitic contamination of vegetables by educating its vendors, farmers and the entire communities/ towns.

869

SOIL-TRANSMITTED HELMINTHIASIS IN ADMITTED CHILDREN WITH SEVERE ACUTE MALNUTRITION, FREETOWN, SIERRA LEONE

Andrew McLellan[1], Lucy Yaguo Ide[2], Lucy Atieno[3], Nellie Bell[3], Mary Hodges[4]

[1]*University of Toronto, Toronto, ON, Canada,* [2]*University of Port Harcourt, Port Harcourt, Nigeria,* [3]*University of Sierra Leone, Freetown, Sierra Leone,* [4]*Helen Keller International Sierra Leone, Freetown, Sierra Leone*

Soil-transmitted helminthes (STH) infection can cause a range of signs and symptoms including abdominal pain, diarrhea, malabsorption, malnutrition, anemia, and impair growth and cognition. STH infections are among the most common infections worldwide and affect the poorest communities. National prevalence of STH infections in school-aged children was 18.5% in 2016, a reduction of 63% since 2008. A survey in two districts in 2016 also found a 19% prevalence of STH infections in pre-schooled aged children (mostly hookworm). These reductions are attributed to two national strategies: biannual mass drug administration (MDA) of Albendazole for children 12-59 months of age since 2006 and the elimination of lymphatic filariasis program that administers both Ivermectin and Albendazole to all above the age 5 years since 2010. The World Health Organization (WHO) recommends MDA twice yearly if prevalence above 50% and annually for prevalence between 20-50%. This study ascertained the prevalence of STH infections in children with a diagnosis based on WHO criteria of Severe Acute Malnutrition (SAM) admitted at the pediatric hospital in Freetown. One stool sample per child aged 6-59 months was collected and examined by a trained laboratory technician within 2 hours of collection using Macroscopy/ Microscopy procedures (Direct Wet Preparation Method). HIV status was collected when results were reported in the medical record. We were unable to carry out any concentration techniques for the detection of cryptosporidium and did not examine more than one sample to adequately rule out giardia. A total of 166 samples were collected. HIV status was reported in 139 medical records with an infection prevalence of 25% (n=35/139). Only one case of STH (*Strongyloides stercoralis*) and no other cysts or protozoa, were found. None of the participant's medical records reported administration of deworming medication upon hospital admission. The study findings suggest that STH infection in children with SAM is uncommon in Freetown and provides additional evidence that STH prevalence is below 20% and bi-annual MDA of pre-school aged children may no longer be necessary.

871

THE *STRONGYLOIDES STERCORALIS*-HOOKWORMS ASSOCIATION AS A PATH TO THE ESTIMATION OF THE GLOBAL BURDEN OF STRONGYLOIDIASIS: A SYSTEMATIC REVIEW

Pedro E. Fleitas[1], Marina Travacio[2], Helena Martí-Soler[3], M. Eugenia Socias[4], Walter R. Lopez[1], Alejandro J. Krolewiecki[1]

[1]*Instituto de Investigaciones de Enfermedades Tropicales (IIET), Universidad Nacional de Salta, San Ramon de la Nueva Oran, Salta, Argentina,* [2]*Universidad de Buenos Aires. Facultad de Farmacia y Bioquímica. Cátedra de Química General e Inorgánica., Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina,* [3]*Universitat de Barcelona, ISGlobal, Hospital Clínic, Barcelona, Cataluña, Spain., Barcelona, Spain,* [4]*University of British Columbia, British Columbia Centre on Substance Use. Department of Medicine, Vancouver, BC, Canada*

Soil-transmitted helminths (STH) represent a significant public health problem. However, until 2020 *Strongyloides stercoralis* was not integrated into the control strategy against STH, given limitations to accurately assess its burden. Considering that *S. stercoralis* shares biological and epidemiological characteristics with hookworms, we describe a new approach for an improved estimation of the burden of infections by *S. stercoralis* based on the prevalence and burden of hookworms and the relationship between these species. Asystematic review of publications,

between 2001 and June 2018, reporting prevalence rates for *S. stercoralis* and hookworms was carried out. The data was classified into two categories: 1) "Community", with surveys including all age groups, and 2) "SAC", with surveys limited to school-aged children. The relationship between *S. stercoralis* and hookworms was characterized in order to estimate the global burden of *S. stercoralis* infections. The study is registered in PROSPERO (CRD42019131127). Spearman correlation coefficient between *S. stercoralis* and hookworms was estimated and the global burden of *S. stercoralis*infections was estimated using a regression model. A total of 119 articles were included, and a significant positive correlation between the burden of *S. stercoralis* and hookworms was identified. Spearman's coefficient for Community surveys was 0.94 and for SAC surveys it was 0.63. Regression models were performed for both population groups with and without a quadratic term for hookworm prevalence and adjusting for diagnostic sensitivity (Hight-S and Low-S) and WHO region. For both populations, the model was superior using High-Ssurveys than all surveys. Based on a linear model, the global burden of *S. stercoralis* infections was estimated at 386 million (95%CI 324 - 449 million) people, including 22 million (95%CI 20 - 24 million) SAC. In conclusion, the significant relationship between *S. stercoralis* and hookworms allows estimating the global burden of *S. stercoralis* infections in most epidemiologic settings using hookworm burden and justifies the search of integrated control activities.

872

SOIL-TRANSMITTED HELMINTHS DIAGNOSTICS PERFORMANCE IN A LOW INTENSITY SETTING AND ITS IMPLICATION ON PROBABILITY OF INFECTION PREDICTION

Berta Grau-Pujol[1], Javier Gandasegui[1], Emanuele Giorgi[2], Helena Martí[1], Valdemiro Escola[3], Anelsio Cossa[3], Jorge Cano[4], Maria Martinez Valladares[5], Jose Muñoz[1]

[1]*Barcelona Institute for Global Health (ISGlobal), Barcelona, Spain,* [2]*CHICAS Research Group, Lancaster Medical School, Lancaster University, Lancaster, United Kingdom,* [3]*Centro de Investigação em Saúde da Manhiça (CISM), Manhiça, Mozambique,* [4]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [5]*Instituto de Ganadería de Montaña (CSIC-Universidad de León), Leon, Spain*

Some regions have successfully reached low prevalence of soil-transmitted helminths (STH). Thus, their programs aim to interrupt transmission and seek confirmation to stop deworming. For that, diagnostic performance verification in low intensity settings is essential to avoid hidden transmission and should be considered in modelling that guides evidence-based program decisions. This study aims to evaluate how the different diagnostic methodologies assess and predict STH infection in a low intensity setting. We conducted a cross-sectional study with 819 participants in Manhiça district, Mozambique. A regular sampling design was used to obtain spatial representativeness. Two stool samples from two consecutive days were collected from each participant. For prevalence, Telemann and Kato-Katz was used; and for intensity of infection, Kato-Katz. We calculated sensitivity of Telemann from one and two samples, single and duplicate Kato-Katz from one sample and, single and duplicate Kato-Katz from two samples using a composite reference standard. Fecal egg counts agreement and Lin's concordance correlation coefficient were assessed. PCR is being performed. Negative binomial generalized linear models were fitted to predict prevalence for each STH using environmental, demographical, socioeconomical, and water and sanitation indicators as predictors. Fifteen per cent of participants were infected with at least one STH by single Kato-Katz from one sample. More than 70 % had a low intensity of infection of ascaris, and >90% of trichuris and hookworm by single Kato-Katz from one sample. The highest sensitivity was observed by Telemann from two samples followed by duplicate Kato-Katz from two samples. Single Kato-Katz from one sample and duplicate Kato-Katz from one sample had the highest agreement. Predicted district maps built from the different diagnostics differed. In conclusion, prevalence determined by the widely used single Kato-Katz from one sample in low intensity settings could be underestimated. Hence, this reduces their infection probability and, thus, guided interventions could be insufficient in those areas.

873

PREVALENCE OF INFECTION WITH SOIL-TRANSMITTED HELMINTHS AND ITS RESPONSE TO TREATMENT WITH ALBENDAZOLE AND MEBENDAZOLE AMONG PARTICIPANTS OF A PFSPZ-VACCINE CLINICAL TRIAL ON BIOKO ISLAND, EQUATORIAL GUINEA

Martin Eka Ondo Mangue[1], Vicente Urbano Nsue Ndong[1], Maria Silvia Angue Lopez Mikue[1], Thabit Mbaga[2], Kamaka Ramadhani Kassim[2], Beltran Ekua Ntutumu[1], Wonder Philip Phiri[3], Guillermo Garcia[3], Maximillian Mpina[4], Peter Billingsley[5], Claudia Daubenberger[4], said Jongo[2]

[1]*Ministry of Health and Social Welfare, Malabo, Equatorial Guinea,* [2]*Ifakara Health Institute, Dar es salaam, United Republic of Tanzania,* [3]*Medical Care Development International, Malabo, Equatorial Guinea,* [4]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [5]*Sanaria Inc, Rockville, MD, United States*

The main soil transmitted helminths (STHs), namely *Ascaris lumbricoides, Necator americanus, Ancylostoma duodenale* and *Trichuris trichiura*, are responsible for the highest health burden among all neglected tropical diseases. The World Health Organization estimates that more than 1.5 billion people worldwide are affected by these infections, the majority of them are children. Although Bioko Island, Equatorial Guinea, has climatic conditions ideal for STH, there is no up-to-date information to estimate the magnitude of the problem that can help design effective control strategies. Such information could be utilized in the planning stage of the PfSPZ vaccine phase III trial in describing the health status of the study population. To investigate the prevalence of soil-transmitted helminths and its response to albendazole and mebendazole treatment among the participants of PfSPZ-Vaccine clinical trials on Bioko Island. This was a cross-sectional study using data from three clinical trials carried out between 2014 to 2018 to evaluate the safety and efficacy of PfSPZ-Vaccine. The study population was 505 healthy Equatoguinean males and females aged between 6 months to 50 years[, residents on the island. The overall prevalence of STH was 42% and was significantly higher among males (71.7%) than in female participants (28.3%). The most prevalent helminth was *T. trichura* (25.7%), followed by *A. lumbricoide* (16.8%) and multiple STH infections (24%). A treatment response rate of both albendazole and mebendazole dose regime within 30 days after treatment was more than 75%, although a response rate of albendazole was a little bit higher (84.6%) compared to that of mebendazole (76.1%). In conclusion, our study showed that the prevalence of STH infections amongst the population of Bioko Island is high and remains a significant health problem. Albendazole and mebendazole dose regimes had a treatment response rate > 75%, implying that a community-based deworming program using both drugs could prove effective.

874

INTESTINAL PARASITE INFECTION WITH A FOCUS ON SOIL-TRANSMITTED HELMINTHS IN AN INDIGENOUS COMMUNITY FROM PUERTO IGUAZÚ (MISIONES), ARGENTINA AND ENVIRONMENTAL AND SOCIOECONOMIC VARIABLES

Ernesto Candela Sentí[1], Carolina Goizueta[2], Carla Muñoz-Antoli[1], **Maria V. Periago**[3]

[1]*Departamento de Biología Celular y Parasitología, Facultad de Farmacia, Universidad de Valencia, Valencia, Spain,* [2]*Fundación Mundo Sano, Buenos Aires, Argentina,* [3]*CONICET, Fundación Mundo Sano, Buenos Aires, Argentina*

Intestinal parasite infections are among the most common worldwide and affect the poorest and most deprived communities, being indigenous communities and children the most vulnerable. The main risk factors for their incidence are related to the lack of access to water and basic

sanitation, poor hygiene and housing conditions. Recent studies in indigenous villages of the province of Misiones (Argentina) have documented high prevalence of infections by a group of intestinal parasites, namely soil-transmitted helminths (STHs). Quantification of infection is important since the pathologies they cause are directly related burden, yet previous studies did not measure intensity. The aim of this study was to determine the prevalence of intestinal parasites, including STHs, and their intensity in an indigenous community from Puerto Iguazú. The Iryapú community is composed of around 100 families of the Mbya-Guaraní ethnicity. The houses were georeferenced and characterized through the use of a standardized questionnaire in which demographic (age, sex, education level) and household variables (construction material, water supply) were studied. Stool samples were processed through sedimentation and Baermann techniques. Samples positive for STHs, except for those with mono-infections by *S. stercoralis*, were thoroughly processed with a Kato-Katz slide. The prevalence of intestinal parasites was 94.3% (133/141) and 78% for STHs (110/141), being hookworm the most prevalent parasite (86.4%) with a prevalence of *S. stercoralis* of 40.9% and of 20% for *A. lumbricoides*. Most of the houses are built with wood and have dirt floors (53.9%), practically the entire population walks barefoot (93.5%) and dispose their excreta in an uncovered pit latrine (93.5%). Almost 80% of the population obtains water for daily use from borehole or wells. The study has detected a hyperendemic area for STHs and poor hygienic conditions that can promote endemicity. Mass deworming programs with albendazole and ivermectin, as well as preventive measures such as health education, are necessary in this area in order to decrease the prevalence and intensity of STH infections.

875

SOIL SURVEILLANCE FOR MONITORING SOIL TRANSMITTED HELMINTHS IN ENDEMIC COMMUNITIES IN INDIA AND BENIN: EXPERIENCE WITH QPCR AND DDPCR

Malathi Manuel[1], Nils Pilotte[2], Joel Thamburaj[1], Rajeshkumar Rajendiran[1], Gokila Palanisamy[1], Saravanakumar P. Kaliappan[1], Joel Edoux Siko[3], Parfait Houngbegnon[4], William E. Oswald[5], David S. Kennedy[5], Maya Nadimpalli[6], Sean R. Galagan[7], Adrian J. Luty[8], Moudachirou Ibikounle[3], Judd L. Walson[7], Steven A. Williams[2], Sitara S.R. Ajjampur[1], Amy J. Pickering[6]

[1]*Christian Medical College, Vellore, India,* [2]*Department of Biological Sciences, Smith College, Northampton, MA, United States,* [3]*Faculté des Sciences et Techniques, Université d'Abomey-Calavi, Abomey-Calavi, Benin,* [4]*Institut de Recherche Clinique du Benin, Abomey-Calavi, Benin,* [5]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [6]*Department of Civil and Environmental Engineering, Tufts University, Medford, MA, United States,* [7]*University of Washington, Seattle, WA, United States,* [8]*Université de Paris, MERIT, IRD, F-75006, Paris, France*

Monitoring the prevalence of soil transmitted helminths (STH) in endemic countries to determine the effect of mass drug administration (MDA) programs is usually carried out with time-consuming, microscopy-based stool surveys. Environmental surveillance with highly sensitive molecular tools can potentially be used as an alternate approach for monitoring STH in these communities. In this pilot study, we developed a sensitive molecular assay for the detection of STH in large volume soil samples and field-tested the protocol. The field sites in India and Benin were part of an ongoing trial on interrupting transmission of STH (DeWorm3). Soil samples were collected from approximately 100 households at the house entrance and near the household water source. DNA was extracted directly from soil samples (20g) and tested by multi-parallel qPCR for 6 species of STH. A total of 153 and 161 soil samples were collected from India and Benin respectively. In India, the overall prevalence of STH by qPCR was 39.9% with a predominance of hookworm (36.6% by qPCR). The overall prevalence in Benin was 34.8% by qPCR with a predominance of *Ascaris* (25.5% by qPCR). Comparisons between qPCR and light microscopy for each STH species suggested that qPCR was more sensitive, and also highlighted issues with misidentification by microscopy. In India, a subset of 40 samples was also tested by droplet digital PCR (ddPCR) for *N. americanus,* which detected a greater number of positive samples at a concentration of 100ng of DNA compared to qPCR. Correlations between soil STH and STH levels in matched human stool samples collected from the same household will be presented. Our results will demonstrate the potential for environmental STH detection to be a useful monitoring tool for population STH infection prevalence that would not require sampling of humans and potentially allow for more representative sampling in communities.

876

SOIL TRANSMITTED HELMINTHIASIS IN RURAL SCHOOL CHILDREN IN RETALHULEU, GUATEMALA

Rebeca E. Vergara Greeno[1], Sainikhil Sontha[2], Richard A. De Los Santos Abreu[3], Marcos D. Lopez Quevedo[4], Emily Almendarez[5], Lisa M. Harrison[1], Lourdes Ralda de Morataya[6], Michael Cappello[1]

[1]*Yale School of Medicine, New Haven, CT, United States,* [2]*Broad Institute of MIT and Harvard, Cambridge, MA, United States,* [3]*Johns Hopkins University, Baltimore, MD, United States,* [4]*Universidad de San Carlos de Guatemala, Guatemala City, Guatemala,* [5]*Yale Univerrsity, New Haven, CT, United States,* [6]*Distrito de Alto Rendimiento, Retalhuleu, Guatemala*

Soil-transmitted helminthiases (STH) are amongst the most common diseases of poverty worldwide, despite extensive Mass Drug Administration (MDA) to school age children. Benzimidazole anthelminthics have variable efficacy against the three most common STHs (*Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm), and there are concerns about emerging resistance. Guatemala is endemic for STH, although the regional distribution of each helminth species is not well defined. Our study's aim was to determine baseline prevalence and intensity of STH infections in children living in Retalhuleu, Guatemala and to evaluate the efficacy of single dose albendazole. We conducted a cross-sectional study of 557 children ages 4-16 from 7 high-risk schools that have implemented biannual deworming. Pre-treatment stool samples were examined via Kato Katz microscopy. Infected children were treated with a single dose of 400 mg albendazole and re-examined 10-20 days post-treatment. The overall prevalence of STH infection was 22.6%, with *T. trichiura* alone (16.7%) being more prevalent than *A. lumbricoides* alone (1.4%) and *T. trichiura/A. lumbricoides* co-infection (4.1%). Hookworm infection was not observed in any study subject. Prevalence of STH infection by school ranged from 7.5% to 42.9% (DAR: 10.5%; Berea Vaquilito: 7.5%; Concepción Ocosito: 42.9%; Lo De Mota: 33.3%; Marina del Rey: 30.3%; San Jose Las Flores: 37.2%; 3 de Enero: 16.1%.) Most infections (99.2%) were light to moderate intensity. Post-treatment cure rates were as follows: 100% (9/9) for *A. lumbricoides*, 23.3% (21/90) for *T. trichiura* and 4.5% (1/22) for *T. trichiura/A. lumbricoides* co-infections. Community-wide fecal egg reduction rates were 99.9% and 50.8% for *A. lumbricoides* and *T. trichiura*, respectively. Our findings confirm poor effectiveness of single dose albendazole for trichuriasis among children in Guatemala. These data support the need for monitoring endemic communities subjected to MDA. Further studies are necessary to identify factors associated with albendazole response and determine optimal dosing regimens in communities with high prevalence of *T. trichiuris*.

877

A RECOMBINASE POLYMERASE AMPLIFICATION TEST FOR STRONGYLOIDES STERCORALIS: MOLECULAR EPIDEMIOLOGY OF STRONGYLOIDIASIS IN TROPICAL VILLAGES IN CUSCO-PERU

Melinda B. Tanabe

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

Strongyloidiasis is the most neglected of the soil-transmitted helminth infections. The overall prevalence of strongyloidiasis is unknown. However, available diagnostic methods are poorly sensitive. Isothermal PCR tests are sensitive alternatives for the diagnosis of neglected parasites. Our objective was to develop a recombinase polymerase amplification lateral flow assay test (RPA-LF) to detect *Strongyloides stercoralis* in the stool. We collected stool samples in three communities (Putucusi, Alto Putucusi, and San

Martin) in the jungle of Cusco, Peru. Samples were immediately tested for *S. stercoralis*larvae using the Baermann's and agar plate culture methods. Formalin preserved stool was tested by rapid sedimentation and ethanol preserved samples were subjected to DNA extraction for Real-Time PCR testing and RPA-LF development. In total, 267 stool samples were tested using the five methods and were used for the analysis. The Strongyloides PCR and RPA-LF had no cross-reactivity with other intestinal parasites. The RPA-LF showed an analytical limit of detection of 20 pg of S. stercoralis DNA extracted from parasites enriched by sedimentation of fresh samples. When compared to a microscopy composite gold standard of Baermann's, rapid sedimentation, and agar plate culture, the RPA-LF had a sensitivity of 87.5% (95% CI 74.5 - 100) and specificity of 59.1% (95% CI 52.6 - 65.6). When compared to a composite of microscopy and real time PCR, the sensitivity and specificity were 90.3% (95% CI: 78.3 - 100) and 72.2% (95% CI: 65.2 -79.2) respectively. The prevalence of S. stercoralis by the agar plate culture was 22.7% in Putucusi, 23.1% in Alto Putucusi, and 29.2% in San Martin. In contrast, the prevalence by Strongyloides RPA-LF was 46.7%, 49%, and 45.1% for each community respectively. The Strongyloides RPA-LF is a novel fast, highly sensitive and specific molecular method. It has the potential to be deployed as a point of care test in remote areas or in low resource settings of endemic countries.

878

SOIL-TRANSMITTED HELMINTH INFECTIONS AND NUTRITIONAL STATUS AMONG SCHOOLCHILDREN IN LAGUNA PROVINCE, THE PHILIPPINES

Mary Lorraine S. Mationg[1], Gail M. Williams[2], Veronica Tallo[3], Remigio Olveda[3], Eindra Aung[1], Portia Alday[3], Mark Donald Reñosa[3], Chona Mae Daga[3], Jhoys Landicho[3], Maria Paz Demonteverde[3], Eunice Dianne Santos[3], Thea Andrea Bravo[3], Franziska A Angly Bieri[1], Yuesheng Li[4], Archie CA Clements[5], Peter Steinmann[6], Kate Halton[7], Donald Stewart[1], Donald P. McManus[4], Darren J. Gray[1]

[1]*The Australian National University, ACT, Australia,* [2]*School of Public Health, University of Queensland, Brisbane, Australia,* [3]*Research Institute for Tropical Medicine, Manila, Philippines,* [4]*Molecular Parasitology Laboratory, Infectious Diseases Division, QIMR Berghofer Medical Research Institute, Brisbane, Australia,* [5]*Faculty of Health Sciences, Curtin University, Perth, Australia,* [6]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [7]*School of Public Health and Social Work, Queensland University of Technology, Brisbane, Australia*

The soil-transmitted helminths (STH) are a significant public health problem in the Philippines, particularly among school-aged children. Children who are infected may suffer from profound physical deficits, including anaemia, malnutrition, stunted growth, cognitive delays and reduced fitness. Although, several large intiatives have been launched to control transmission, STH remains highly endemic in the Philippines. To investigate the impact of STH infections on children's health, we used the baseline cross-sectional data collected from 40 schools included in the "Magic Glasses Philippines" randomised controlled trial in Laguna Province, the Philippines. Analyses of the association between STH infections and haemoglobin and child development indices such as stunting and wasting were undertaken. Stool samples were collected from approximately 2000 schoolchildren and assessed for STH infections using Kato-Katz thick faecal smear microscopy technique. Participants' data on haemoglobin, height and weight were recorded. Questionnaires were employed to collect demograpic and socio-economic data. Here we will detail the results of this investigation. This study will provide basis in the formulation of strategies to improve child development and morbidity outcomes in this setting.

879

A LONGITUDINAL ANALYSIS OF DATA FROM TWO COHORTS SHOWS THAT MASS DRUG ADMINISTRATION OF ALBENDAZOLE FOR FILARIASIS ELIMINATION IN CENTRAL AFRICA HAS A DOSE-RELATED BENEFICIAL EFFECT ON SOIL-TRANSMITTED HELMINTHS

Jérémy T. Campillo[1], Naomi P. Awaca-Uvon[2], Jean-Paul Tambwe[3], Godé Kuyangisa Simuna[3], Gary J. Weil[4], Michel Boussinesq[1], Cédric B. Chesnais[1], Sébastien D. Pion[1]

[1]*Institut de Recherche pour le Développement, Montpellier, France,* [2]*Ministry of Health, Kinshasa, Democratic Republic of the Congo,* [3]*Ministry of health, Kinshasa, Democratic Republic of the Congo,* [4]*Washington University School of Medicine, St. Louis, MO, United States*

The impact of semi-annual mass drug administration (MDA) with albendazole alone (ALB 400 mg) on lymphatic filariasis (LF) and soil-transmitted helminth (STH) infections was assessed during two trials conducted from 2012 to 2019 in the Republic of Congo and Democratic Republic of Congo. The data collected were reanalyzed to evaluate the effect of individual adherence to ALB treatment on STH infections. Participants were treated 6-monthly but invited to provide a stool sample every year, just before MDA. STH infections were diagnosed with duplicate Kato-Katz thick smears, and results were reported as eggs per gram of stool. All subjects with an STH infection before their first ALB treatment who had at least one follow-up test result were included in the analyses. We used parametric survival models to assess the influence of compliance to ALB treatment on the probability to present a negative Kato-Katz test. Out of 2658 subjects included in the trials, data on 177 persons (620 person-years, PY), 211 (651 PY) and 270 (1013 PY) with hookworm (HW), *Ascaris lumbricoides* and *Trichuris trichiura*, respectively at baseline were available for the analysis. The impact of ALB was overall dose-related for all three STH. For hookworm, the time ratios (i.e. the factor by which the time required to testing negative is multiplied) were 1.55 ($P < 0.001$) and 0.92 ($P = 0.255$) for participants who took 0 and 2 doses per year, respectively, compared to those who took 1 dose per year. For *Ascaris*, the time ratios were 1.60 ($P = 0.002$) and 0.84 ($P < 0.001$) for participants who took 0 and 2 doses per year, respectively, compared to those who took 1 dose per year. For *Trichuris*, the time ratio was not calculable for the 0-dose category. Nevertheless, the time ratio was 0.82 ($P<0.001$) for participants who took 2 doses, compared to those who took 1 dose per year. Overall, ALB was less effective to reduce the prevalence in subjects with moderate or high baseline infection intensities compared to those with light ones. Our results confirm the beneficial effect of ALB MDA provided for LF elimination on STH and document the additional benefits of semi-annual over annual regimens.

880

PREVALENCE, INTENSITY, DISTRIBUTION AND DETERMINANTS OF SOIL-TRANSMITTED HELMINTHS (STH) INFECTION AMONG PRE-SCHOOL AGE CHILDREN AFTER 10 YEARS OF PREVENTIVE CHEMOTHERAPY INTERVENTION: EVIDENCE TO IMPROVE CONTROL STRATEGIES OF STH IN GAMO GOFA ZONE, SOUTHERN ETHIOPIA

Mekuria Asfaw[1], Tigist Gezmu[1], Teklu Wegayehu[1], Alemayehu Bekele[1], Zeleke H/Mariam[1], Nebiyu Masresha[2], Teshome Gebre[3]

[1]*Arba Minch University, Arba Minch, Ethiopia,* [2]*Ethiopian Public Health institute, Addis Ababa, Ethiopia,* [3]*International Trachoma Initiative, Addis Ababa, Ethiopia, Ethiopia*

Pre-school age children (PSAC) pay one of the highest toll of morbidity associated with Soil-transmitted helminths (STH). Preventive chemotherapy (PC) has been implemented in Ethiopia at national level to reduce the prevalence of moderate and heavy infections with STH in PSAC and school age children (SAC) to below 1% by 2020. Effect of PCon STH infection among SAC was revealed by many earlier studies, whereas evidence is lacking on prevalence, intensity, distribution and determinants of STH among PSAC in Gamo Gofazone, southern Ethiopia. The study was

conducted in five district of Gamo Zonefrom November 2018 to January 2019. A mixed study design was used-cross-sectional and case-control studies. Randomly selected PSAC (2462 for cross-sectional and 1206 for case control) were participated . Data were collected using pre-tested questionnaire, and Kato-Katz for laboratory diagnosis of stool samples. Data were entered into Epi data 4.4.2, then exported toSPSS software (IBM, version 25) for analysis where summary statistics, and binary logistic regressions were undertaken. Overall, the prevalence of STH among PSAC was 23.5%,which is moderate by intesity. The highest prevalence of STH was observed in Chencha district,33.76 %, whereas the least prevalence was observed in Demba Gofa district, 10.97 %. All of the infections with hookworms and *Trichuris trichuria* were low by intensity, but 15% of *Ascaris lumbricodes* infections were moderate by intensity. Having no functional hand washing facility and low mean score on knowledge of STH transmission were associated with STH infection; while living in urban area, availability of piped water and walking less than 30 minutes to water source were associated with protection against STH infection. In conclusion, STH is still found as a serious public health problem among PSAC despite PC has been implemented for more thana decade. An urgent call for action is required to adequately implement WASH implementation such as access to safe water, hand washing facility and SBCC (social, behavioural, change and communication) to control and eliminate STH among PSAC in the zone.

881

MUCUS-DEGRADING BACTERIAL SPECIES COULD BE IMPORTANT BIOMARKERS FOR ACTIVE HOOKWORM INFECTIONS: A PILOT STUDY

Jewelna Efua Birago Akorli, Lydia Okyere, Hilda Darko, Michael David Wilson

Department of Parasitology, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana

The human gut microbiome plays significant roles in health and disease. Comprehensive profiles of the gut microbiome during parasitic infections could provide information on parasite infection mechanisms, variations in population disease statuses and could potentially be used as biomarkers. We aim to appreciate the involvement of the human gut microbiome in soil-transmitted helminth (STH) infections and outcome of drug therapy. Stool was collected from study participants in Kintampo North District, Ghana, a hookworm endemic area. Samples used in this pilot experiment were baseline prior to albendazole therapy. To demonstrate whether there are measurable impacts of human gut microbiota on STH infection or vice versa, DNA was extracted from 5 each of *Necator americanus* positive and negative stool samples, confirmed by PCR. The DNA were sequenced for bacterial 16S V2-4-8 variable region using Ion Torrent technology. Sequences were analyzed for bacterial diversity and relative abundance using QIIME 2. Our results indicated that hookworm positive and negative individuals had similar microbial diversity (Shannon-Wiener index: $P>0.05$). *Firmicutes* and *Proteobacteria* phyla were dominant with average relative proportions of 42% and 26.5%, respectively. Although *Proteobacteria* abundance differed between groups ($P= 0.0002$), it was microbiota that belonged to other 'minor' phyla that showed significant differential abundance. Infected individuals had significantly less bacteria belonging to family *Bifidobacteriaceae* ($P< 0.0001$) and *Neisseriaceae* ($P= 0.006$), both natural commensals of healthy human gut. *Kiritimatiellae*, a recently discovered bacteria of the phylum *Verrucomicrobia* was significantly more abundant in infected persons ($P= 0.01$). Interestingly *Akkermansia*, another species of *Verrucomicrobia* is a mucus-degrading bacterium that has been found to be associated with healthy guts. Our preliminary data support the hypothesis that the gut microbiota is dysregulated in the presence of *N. americanus*. The implications of this as biomarkers of active infection and treatment outcome needs further investigation.

882

ASCARIS INDUCED ALLERGIC AIRWAY DISEASE IS MEDIATED BY RAG-INDEPENDENT IMMUNE PATHWAYS

Jill Weatherhead, John Morgan Knight, Maria Elena Bottazzi, David Corry, Peter Hotez

Baylor College of Medicine, Houston, TX, United States

Ascariasis is the most common helminth infection worldwide and accounts for significant global morbidity including a severe allergic airway disease phenotype due to larval lung migration. This study evaluated the role of adaptive immune pathways, specifically by blocking T and B lymphocyte development via Recombination-activating gene (RAG) deficiency, in the development of *Ascaris* induced allergic airway disease. Wild-type (WT) and RAG-1$^{-/-}$ mice were given *Ascaris suum* embryonated eggs by oral gavage. Plethysmography was completed and respiratory system resistance (R_{RS}; cm $H_2O \cdot s \cdot ml^{-1}$) was measured to assess airway hyperresponsiveness (AHR). Bronchoalveolar lavage (BAL) fluid was collected and total cell counts were determined using cytospin and microscopy. Lung tissues were collected for cytokine analysis using Luminex MAGPIX® and histopathology with H&E stain. *Ascaris* infected RAG-1$^{-/-}$ mice had mildly, but not statistically significant, attenuation of AHR compared to infected WT controls. Total larval burdens in the lungs (p=ns) were also similar between *Ascaris* infected WT and infected RAG$^{-/-}$ mice. Increased BAL fluid hemoglobin concentrations and increased total inflammatory cell counts, driven primarily by neutrophils and eosinophils, were similar between *Ascaris* infected WT and RAG$^{-/-}$ mice (p = ns) compared to uninfected controls. *Ascaris* infected WT mice did have significantly increased lymphocyte infiltration compared to infected RAG$^{-/-}$ mice and uninfected controls ($p<0.01$). We furthermore detected *Ascaris-dependent* type-2 cytokine expression, including IL-13, in lung tissue ($p<0.01$) in both infected WT and RAG$^{-/-}$ mice. Histologically, both WT and RAG$^{-/-}$ infected lung tissue had dense eosinophilic infiltrates around bronchovascular bundles. The lack of mature T and B lymphocytes did not impair development of severe, acute *Ascaris*-induced allergic airway disease. Further evaluation of innate immune pathways driving *Ascaris*-induced allergic airway disease, specifically targeting innate lymphoid cells (ILC), will further pathophysiologic insight in order to develop novel interventions.

883

A CRITICAL ANALYSIS OF SOIL TRANSMITTED HELMINTHIASIS IN THE UNITED STATES, PERSISTING NEGLECTED INFECTIONS OF POVERTY

Mary K. Lynn, Josephine A. Morrissey, Donaldson F. Conserve

University of South Carolina, Columbia, SC, United States

Soil transmitted helminthiases (STH) are among the leading neglected tropical infections globally, disproportionally affecting children in the world's most underserved communities. After over a decade of mass drug administration (MDA) the Global Burden of Disease Study 2017 has shown significant reductions in disease prevalence on a global scale. However, in the USA, where MDA is not suggested, a paucity of research has existed in this arena for nearly 40 years, despite an extensive documented history of helminth infections in the American south and Appalachia where rates of hookworm infection have previously reached 40% prior to the 1980's. Suitable environmental conditions for nematode viability in this region, concentrated areas of child poverty that exceed national averages, and inadequate plumbing facilities suggest the potential that these infections may continue to plague children in the region with an array of clinical outcomes including impaired cognition and anemia. The goal of this study was to set the stage for the likely persistence of "the germ of laziness" and other STH infections in children in low-income areas of the southern USA and Appalachia region. We conducted a review and critical analysis of STH in the American southeast and Appalachia gathering evidence from a socioecological perspective to identify high risk hotspots in these regions of the USA for future prevalence studies. We found consistently overlapping areas of these select social and environmental indicators

running through central counties along the southeastern coastal states and counties in close proximity to the Appalachian Mountains in eastern Kentucky and West Virginia. Results of this analysis should be used for further clinical and epidemiologic investigation and to inform public health decision makers for future interventions.

885

MULTI-PARALLEL QPCR TO IDENTIFY STH IN SOIL AND WATER SAMPLES MAY PROVIDE AN ALTERNATIVE TO SAMPLING OF HUMAN STOOL FOR MONITORING OF STH CONTROL PROGRAMS: EXPERIENCE FROM KENYA

Benard Chieng[1], Gretchen Walch[2], Sylvie Araka[1], Nils Pilotte[2], Jenna Swarthout[3], Sitara SR Ajjampur[4], Steven A. Williams[2], Sammy M. Njenga[1], Amy J. Pickering[3]

[1]*Kenya Medical Research Institute, Nairobi, Kenya,* [2]*Smith College, Northampton, MA, United States,* [3]*Tufts University, Medford, MA, United States,* [4]*Christian Medical College, Vellore, India*

Monitoring of soil-transmitted helminth (STH) control programs necessitates frequent testing of endemic populations. Recent advances in molecular testing techniques have improved upon the sensitivity and specificity of microscopy-based diagnostics, however, the collection of human stool remains a challenge in these settings. The testing of individual human stool samples is low throughput and resource intensive, and the collection of human stool carries stigma in many endemic populations. For these reasons, exploring the use of environmental samples, such as soil and water, to improve throughput and eliminate the need for human sampling presents an attractive alternative. However, existing techniques for environmental sampling of STH are microscopy-based. Here we present newly developed and field-tested methods for qPCR-based detection of STH DNA in soil and drinking water. Spiking studies revealed the detection limits of this new method are as follows: *Ascaris suum*: 5 eggs/ 20 grams of soil, *Necator americanus*: 2 eggs/ 20 grams of soil, *Trichuris trichiura*: 10 eggs/ 20 grams of soil, and *Ascaris, Necator, and Trichuris*: 10 eggs/ 4 liters of water. Environmental sampling with this technique was compared with stool sampling among households sampled in Kibera and Dagoretti sub-counties in Kenya; prevalence of any STH were: 59.2% in soil (n=169) and 35.5% in human stool (n=279). All three STH species were detected in soil: *Ascaris* in 58% of community soil samples, hookworm in 20% of soil samples, and *Trichuris* in 8% of soil samples; while no STH species were detected in drinking water samples. *Ascaris* was the most prevalent species in both soil and human stool, with a prevalence of 58.0% in soil and 31.5% in stool. STH prevalence was higher in soil collected from community water points compared to household soil. Future research should investigate if soil STH levels could predict human infection prevalence thresholds with STH species.

886

WASH AND ENVIRONMENTAL RISK FACTORS FOR SOIL-TRANSMITTED HELMINTH PREVALENCE AND INTENSITY OF INFECTION IN THE WESTERN PROVINCE SOLOMON ISLANDS

Brandon Le[1], Naomi Clarke[1], Sze Fui Hii[2], Aisling Byrne[1], Oliver Sokana[3], Alam Khattak[1], Susana Lake[4], Lucia Romani[1], Daniel Engelman[4], Titus Nasi[5], Dickson Boara[6], Rebecca Traub[2], John Kaldor[1], Andrew Steer[4], Susana V. Nery[1]

[1]*The Kirby Institute, University of New South Wales, Sydney, Australia,* [2]*University of Melbourne, Melbourne, Australia,* [3]*Ministry of Health and Medical Services, Honiara, Solomon Islands,* [4]*Murdoch Children's Research Institute, Melbourne, Australia,* [5]*National Referral Hospital, Honiara, Solomon Islands,* [6]*Gizo Hospital, Gizo, Australia*

Soil-transmitted helminth (STH) infections - caused by *Ascaris lumbricoides, Trichuris trichiura,* hookworm (*Necator americanus, Ancylostoma duodenale, Anyclostoma ceylanicum),* and *Strongyloides stercoralis* - affect 900 million people globally. Transmission relies on a complex interaction between favourable environmental conditions and water, sanitation and hygiene (WASH) access and behaviours. There is limited understanding of STH epidemiology in the Solomon Islands, and no published research on STH infections in the Western Province. Furthermore, there is little research on WASH and environmental risk factors for STH infections in the Solomon Islands. A cross-sectional survey of 18 communities in the Western Province of Solomon Islands was conducted in May-July 2019. Stool samples were collected from 830 participants. Prevalence of each STH species and infection intensity of *A. lumbricoides*, *T. trichiura*, and *N. americanus* were determined using quantitative PCR. Demographic and WASH data were collected through individual questionnaires. Environmental data was obtained through open-access sources that use geographic information systems technology. Overall prevalence of hookworm infection across all communities was 58.9% (ranging from 22.5 to 91.5%), while prevalence of *T. trichiura* was 20.7% (ranging from 0 to 79.2%), *S. stercoralis* 5.9% (ranging from 0 to 29.2%), and *A. lumbricoides* 0.7% (ranging from 0 to 16.7%). There was significant heterogeneity in prevalence between communities. We will use generalised linear mixed models to analyse risk factors for infection with *N. americanus*, *T. trichiura*, and *A. ceylanicum*, as well as risk factors for heavy-intensity infection with *N. americanus* and *T. trichiura*. Models will incorporate demographic, environmental, and WASH risk factors and will adjust for clustering at community and household levels. This study contributes to the understanding of STH epidemiology in the Solomon Islands and will extend the literature on the role of WASH behaviours and environmental conditions in contributing to risk of STH infection.

887

FEMALE GARMENT WORKERS UNDERSTANDINGS OF HIV IN BANGLADESH

Shakeel Ahmed Ibne Mahmood

The University of Newcastle, Newcastle, Australia

The proposed objective is to critically examine female garment workers, FGWs experiences and their personal understandings of their human immunodeficiency virus, HIV experiences in Bangladesh. A systematic and comprehensive search conducted on relevant literature on FGWs health on HIV within Bangladesh published between 1989 and 2018. Relevant information from selected articles extracted, presented and attempted to contribute to existing literature in the form of new findings and critically interpret existing findings. Corresponding review of the literature continue to form inherent components of the debate. According to one study in 2013, FGWs are at risk groups in Dhaka City, who are regularly engaged in illegal and unsafe sex. Major causes of HIV vulnerability of FGWs are gender inequality, multiple sex partners, drug abuse and rape violence. These poor FGWs are not informed about contraceptive methods, safe sex, menstruation and hygiene and HIV infection due to low literacy rate. Another study in 2012, FGWs revealed 43% do not use condoms and 95% believes that HIV is treatable. Thus, HIV related risk behaviors' of FGWs might have a substantial impact on the future course of HIV epidemic in Bangladesh. This research has contributed to broader disciplinary knowledge and/or policy practice on STIs prevention within FGWs of Dhaka City. Empowering FGWs through formal health education on HIV is essential, including prevention of workplace violence and intimate partner violence related training. Community leaders, private sector involvement and business in HIV needs to be encouraged. Counselling and information are critical components to support women in making sexual intercourse decisions and carrying them out voluntarily and safely. Currently, surveillance has been only conducted in key population, therefore outcome of this study recommend a large-scale study on the FGWs in urban areas of Bangladesh to guide policymakers and researchers on how to prevent HIV and improve FGWs health.

888

PREVALENCE OF MALARIA AND TYPHOID CO-INFECTION AMONG OUTPATIENTS ATTENDING IJANIKIN HEALTH CENTER, IJANIKIN, LAGOS STATE, NIGERIA

Jacob Babasola Ajayi[1], Oluseyi Abike Ajayi[2]

[1]*Ogun State Institute of Technology, Igbesa, Ogun State, Lagos, Nigeria,* [2]*Lagos State University, Lagos, Nigeria*

Malaria and salmonella infections are endemic especially in developing countries; however malaria and salmonella co-infection is a rare entity with high mortality. An association between malaria and typhoid fever was first described in 1862 in North America as an entity called typho-malaria fever. A study was carried out on patients clinically diagnosed of malaria and typhoid at Otto Ijanikin health center, Lagos State, Nigeria to verify the degree of relationship between malaria and typhoid fever. A total number of 200 patients were sampled. Widal kit and Rapid diagnostic were used for typhoid and malaria diagnoses respectively. *Plasmodium falciparum* was the only parasite used to indicate the presence of malaria in the patients. The study indicated that out of 200 patients, 50 (25%) were positive for malaria of which 16(32%) were male while 34 (68%) were female. A total number of 69 patients were positive for typhoid of which 24 were male and 45 were female. Out of 16 male with malaria, 10(62.5%) were typhoid positive and out of 34 female positive for malaria, 18(52.9%) were positive for typhoid infection. In conclusion, There is no positive relationship between malaria and typhoid ($p < .05$). The results also indicate that the differences in plasmodium falciparum parasiteamia and the antibody titre of Salmonnela is significant in using the Widal test.

889

ANTI-RETROVIRAL TREATMENT WAS ASSOCIATED WITH CARDIOVASCULAR DISEASE RISK FACTORS BUT NOT WITH ENDOTHELIAL DYSFUNCTION IN HIV PATIENTS IN MTHATHA, EASTERN CAPE PROVINCE OF SOUTH AFRICA

Godwill Azeh Engwa, Benedicta Nkeh-Chungag

Walter Sisulu University, Mthatha, South Africa

Though some findings have reported increased cardiovascular disease (CVD) risk in HIV persons on antiretroviral therapy (ART) in South Africa, the limited information on the effect of ART on vascular function in this population was the rational for this study. It was therefore the interest of this study to investigate the relationship between ART and vascular function as well as CVD risk factors in South Africans with HIV. This was a cross-sectional study that recruited HIV subjects in Mthatha, Eastern Cape Province of South Africa. Anthropometric and blood pressure parameters were measured. Lipid profile, glycaemic indices, $CD4^+$ count, HIV viral load and albumin to creatinine ratio (ACR) were assayed in blood. Flow mediated dilation (FMD) test was performed to assess endothelial function. A total of 480 participants were recruited which included 146 subjects on anti-retroviral therapy (ART), 163 HIV subjects not on ARV (HIV naive) and 171 subjects without HIV. Measures of obesity (waist circumference and waist to hip ratio) were significantly ($p<0.05$) higher in HIV subjects while HDL level was significantly ($p<0.05$) increased in HIV subjects on ART. Increased ACR level was significantly ($p<0.05$) higher in HIV subjects, particularly in HIV naive subjects. FMD was insignificantly ($p>0.05$) different between subjects with HIV and those without HIV. Moreover, the 3rd quartile of FMD showed a significantly ($p<0.05$) higher level of cholesterol and LDL in hypertensive HIV subjects on ART suggesting ART to promote dyslipidaemia. Obesity and increased ACR were associated with HIV. There was a positive relationship between FMD and CD4 count on HIV subject on ART suggesting that ART may improve endothelial function in HIV subjects. In conclusion, ART was associated with renal-cardiovascular risk in HIV patients and hypertension was shown to be a contributory factor to the cardiovascular risk factors. Though ART was not associated with endothelial dysfunction, it could possibly ameliorate endothelial function in HIV subjects.

890

INTRACARDIAC TUBERCULOMA IN THE IMMUNE-COMPROMISED POPULATION - A RE-VISITATION

Cornelius C. Nwora

Texas Southern University, Houston, TX, United States

Tuberculosis (TB) is a contagious and an airborne disease. The World Health Organization (WHO) ranks TB alongside HIV/AIDS as a leading cause of death worldwide.[1]Cardiac tuberculosis is usually found at postmortem examination. Endocardial tuberculoma is extremely rare, found in only 0.14% of autopsy cases. Studies carried out before the introduction of specific anti-tuberculous therapy assert that the myocardium is involved in less than 0.30% of patients dying of tuberculosis.[2] The myocardium might be affected by direct spread from the mediastinal gland, by the lymphatic routes, or the bloodstream. Organ involvement with M. tuberculosis infection was classified as being miliary (numerous small tubercles each less than 3 mm in diameter and resulting from blood-spread infection) or nodular (large rounded tuberculous lesions formed by confluent foci of tuberculous infection). Nodular myocardial tuberculosis might develop into a ventricular aneurysm.[3] About 4% of children infected under the age of 5 years, develop tubercular meningitis or miliary TB.[4]Among immunocompetent adults, miliary TB accounts for less than 2% of all cases of TB and up to 20% of all extra-pulmonary TB (EPTB) cases in various clinical studies.[4] However, EPTB accounts for more than 50% of all cases of TB in late HIV infection.[5] In the pre-antibiotic era, miliary TB was predominantly a disease of infants and children. During the last three decades, it is increasingly being recognized in adults as well, and several reasons are thought to be responsible for this changing epidemiological trend including: HIV/AIDS, ever increasing list of immunosuppression, such as use of biologicals and immunosuppressive drugs for treatment of various medical disorders, increasing occurrence of organ transplantation, chronic haemodialysis program, among others.[6] Currently, two peaks are evident - one involving adolescents and young adults and another later in life among elderly persons.[7] According to WHO's 2015 Global Tuberculosis Report, 9.6 million people fell ill with TB in 2014, including 1.2 million people living with HIV.

890

INTRACARDIAC TUBERCULOMA IN THE IMMUNE-COMPROMISED POPULATION - A RE-VISITATION

Cornelius C. Nwora

Texas Southern University, Houston, TX, United States

Tuberculosis (TB) is a contagious and an airborne disease. The World Health Organization (WHO) ranks TB alongside HIV/AIDS as a leading cause of death worldwide.[1]Cardiac tuberculosis is usually found at postmortem examination. Endocardial tuberculoma is extremely rare, found in only 0.14% of autopsy cases. Studies carried out before the introduction of specific anti-tuberculous therapy assert that the myocardium is involved in less than 0.30% of patients dying of tuberculosis.[2] The myocardium might be affected by direct spread from the mediastinal gland, by the lymphatic routes, or the bloodstream. Organ involvement with M. tuberculosis infection was classified as being miliary (numerous small tubercles each less than 3 mm in diameter and resulting from blood-spread infection) or nodular (large rounded tuberculous lesions formed by confluent foci of tuberculous infection). Nodular myocardial tuberculosis might develop into a ventricular aneurysm.[3] About 4% of children infected under the age of 5 years, develop tubercular meningitis or miliary TB.[4]Among immunocompetent adults, miliary TB accounts for less than 2% of all cases of TB and up to 20% of all extra-pulmonary TB (EPTB) cases in various clinical studies.[4] However, EPTB accounts for more than 50% of all cases of TB in late HIV infection.[5] In the pre-antibiotic era, miliary TB was predominantly a disease of infants and children. During the last three decades, it is increasingly being recognized in adults as well, and several reasons are thought to be responsible for this changing epidemiological trend including: HIV/AIDS, ever increasing list of immunosuppression, such as use of biologicals and immunosuppressive

drugs for treatment of various medical disorders, increasing occurrence of organ transplantation, chronic haemodialysis program, among others.[6] Currently, two peaks are evident - one involving adolescents and young adults and another later in life among elderly persons.[7] According to WHO's 2015 Global Tuberculosis Report, 9.6 million people fell ill with TB in 2014, including 1.2 million people living with HIV.

891

PREVALENCE OF TB/HIV CO-INFECTION AMONG PATIENTS ATTENDING HIV CLINIC IN JUBA TEACHING HOSPITAL, SOUTH SUDAN: FIVE YEARS RETROSPECTIVE STUDY, JANUARY 2010 - DECEMBER 2014

Richard Lino Loro Lako[1], Kenneth Sube[2], Anthony Lasuba[2], Joseph Lako[2], Salvador Jaja[2], Charles Longoya[3]

[1]*Ministry of Health, Juba, South Sudan,* [2]*College of Medicine, University of Juba, Juba, South Sudan,* [3]*Royal Infirmary of Edinburgh, Dept of Infectious Dis and Medical Microbio, Edinburgh, United Kingdom*

The Ministry of Health of South Sudan assessment of the prevalence of TB/HIV co-infection in the country in 2010 put it at 9.7% nationally while the United Nations Development Program (2010) in South Sudan had earlier estimated at the various facilitates across the country a range from 10% to 20%. The aim of this retrospective study was to determine the prevalence of tuberculosis among HIV positive adult patients in the HIV clinic in Juba Teaching Hospital in South Sudan. Data from registers of HIV positive patients for the period 2010 - 2014 were reviewed. The socio-demographic, treatment and clinical status of participants were determined and described using percentages and frequencies for categorical data while means and standard deviations were used for describing the continuous data. The Analysis of Variance (ANOVA) or t-test was used for making comparisons while the Pearson's correlation coefficient test was used to evaluate associations in continuous variables and the Chi squared test was used for categorical variables. The level of significance was set at $p< 0.05$. Out of 183 patients, 110 (60.1%) were females and 73(39.9%) were males. TB/HIV co-infection prevalence was 19.6% and the mean age of the co-infected was 34.2 (SD +/-10.38) years. Out of the co-infected, 28 (62.2%) were females and 17(37.8%) males. No significant sex association was observed ($p=0.7$) among co-infected. The co-infection rate was significantly higher ($p=0.02$) among unmarried/single patients. The year of diagnosis of TB among HIV patients showed significant association ($p=0.01$) with 2013 having the highest prevalence (24.6%) compared to the other years. Prevalence of TB is high among HIV positive patients, and young/unmarried people with positive HIV status are more likely to have TB infections.

892

ANTIBODIES TO *PLASMODIUM FALCIPARUM* ANTIGENS AND THE ASSOCIATION OF ANEMIA AMONG PEOPLE LIVING WITH HIV IN WESTERN KENYA

Eliud O. Odhiambo[1], Dibyadyuti Datta[2], Katrina Co[2], Lindsey B. Turnbull[2], George Ayodo[3], Sarah E. Cusick[4], Chandy C. John[2], Anne EP. Frosch[5]

[1]*Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN, United States,* [2]*Ryan White Center for Pediatric Infectious Diseases & Global Health, Indiana University School of Medicine, Indianapolis, IN, United States,* [3]*Center for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya,* [4]*Department of Pediatrics, University of Minnesota Medical School, Minneapolis, MN, United States,* [5]*Hennepin Healthcare Research Institute, Minneapolis, MN, United States*

Major causes of anemia in HIV include inflammation, opportunistic infection, and iron deficiency. However, in malaria-endemic areas, malaria may disproportionality drive anemia in people living with HIV (PLWH) compared to HIV-negative (HN) individuals. Malaria causes anemia through hemolysis, bone marrow suppression, and antibody-mediated phagocytosis of malaria-infected RBCs (iRBCs). We recently found that malaria-specific antibodies are higher in PLWH compared to HN individuals in Bondo, Kenya. It is possible that during malaria infection, PLWH would experience greater RBC loss secondary to antibody-mediated iRBC phagocytosis. To generate preliminary data for this hypothesis, we examined whether increased malaria antibodies were associated with anemia in healthy PLWH and HN individuals. We measured IgG, IgM, and IgG subclass (IgG1-4) antibodies against 2 *P. falciparum* antigens, AMA-1 and GLURP-R0, in 181 adults (129 PLWH, 52 HN). In each group, seroprevalence and antibody levels were compared in people with and without anemia [defined by a hemoglobin (Hgb) level <12g/dL (women) or <13g/dL (men)]. Among PLWH, the proportion of participants with positive IgM and IgG3 antibody to AMA-1, and IgG1 to GLURP-R0 was higher in anemic compared to non-anemic individuals (62% vs 31%; 94% vs 79%; 79% vs 54% respectively; all $p<0.05$). Correspondingly, antibody levels (arbitrary units) were higher for anemic vs. non-anemic PLWH individuals for IgM and IgG1 to AMA-1 (1.12 vs 0.93; 1.80 vs 1.47; all $p<0.05$), and IgG1 and IgG3 to GLURP-R0 (1.41 vs 1.04; 3.08 vs 2.39; all $p<0.05$). Among HN individuals, the proportion of people with a positive IgG2 antibody to GLURP-R0 was lower in anemic compared to non-anemic individuals (0% vs 29%; $p=0.04$), and IgG2 levels to GLURP-R0 were lower in anemic than non-anemic individuals (0.75 vs 0.86; $p=0.02$). In conclusion, specific IgM and cytophilic subclass antibodies to AMA-1 and GLURP-R0 were associated with increased likelihood of anemia in PLWH but not in HN. Future studies should assess whether these antibodies are associated with a larger drop in Hgb in PLWH during acute malaria infection.

893

PERFORMANCE OF THE HAIN GENOTYPE MTBDRSL ASSAY FOR RAPID DETECTION OF EXTENSIVELY DRUG-RESISTANT TUBERCULOSIS IN BAMAKO, MALI

Antieme Combo Georges Togo, Hawa n'baye HBD Drame, Boureima Degoga, Bassirou Diarra, Bocar Baya, Michael Belson, Sounkalo Dao, Seydou Doumbia

SEREFO/UCRC, Bamako, Mali

Molecular tests for detecting drug resistant tuberculosis, such as the Genotype® MTBDR*sl* assay, have shown promising results for diagnosis of MDR-TB. Although the prevalence of MDR-TB and associated genetic mutations in Mali are not well documented, little information on extensively drug-resistant and its characteristics exist. Hence, the evaluation of pre-XDR and XDR prevalence, as well as the mutation status of *gyrA, gyrB, rrs*, and *eis* promoter region, associated with resistance to second-line drugs, would be of great interest. To determine the frequency of mutations associated with resistance to fluoroquinolones and second-line injectable drugs (SLIDs), in culture isolates confirmed as *M. tuberculosis* complex in Bamako. Between November 2016 and December 2019, we performed a cross sectional study to enroll new and previously treated TB patients for the detection of mutations associated with second-line drugs. Patients were first screened with Xpert MTB/RIF, and conventional BACTEC MGIT/AST/SIRE drug susceptibility testing (DST). Thereafter rifampicin resistant (RR), and/or MDR/TB patients were tested with LPA MTBDRsl for mutation in the specific genes of fluoroquinolones (*gyrA and gyrB genes*) and second-line injectable drugs (SLID) (*rrs and eis genes*). Among the 573 patients screened, the prevalence of RR, and/or MDR/TB using Xpert MTB/RIF and conventional DST was 12.74% (73/573). Thus, the 73 RR/MDR isolates were tested with MTBDRsl assay and 9.6% (7/73) showed resistance to at least one second line drug and tow isolates (2.74%) were XDR. 7% of patients were co-infected with HIV, and 86.30% were diseased with MTB-Lineage 4. Genotypic analysis revealed that two XDR strains harbored mutations in the *gyrA gene* and one isolate has mutations in the *rrs gene.* No mutation was found in *gyr*B and *eis* genes. In conclusion, most of mutations associated with SLD resistance occurred in *gyrA* gene and *rrs* gene. These findings highlight the impact of mutations in *gyrA* in the development of fluoroquinolones resistance and provide the first estimates of the proportion of pre-XDR-TB among MDR-TB cases in Mali.

894

LATENT CO-INFECTIONS INCREASE RISK OF NEUROCOGNITIVE IMPAIRMENT AMONG OLDER PERSONS WITH HIV IN LIMA, PERU

Monica M. Diaz[1], Marcela Gil Zacarías[2], Maisie A. Bailey[3], Donald Franklin[4], Mariana Cherner[5], Serggio Lanata[6], Ronald J. Ellis[7], Patricia J. Garcia[8]

[1]*Dept of Medicine, University of California, San Diego, San Diego, CA, United States,* [2]*Facultad de Medicina Alberto Hurtado, Universidad Peruana Cayetano Heredia, Lima, Peru,* [3]*Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States,* [4]*HIV Neurobehavioral Research Center, University of California, San Diego, San Diego, CA, United States,* [5]*Dept of Psychaitry, University of California, San Diego, San Diego, CA, United States,* [6]*Weill Institute for Neurosciences, Memory and Aging Center, University of California, San Francisco, San Francisco, CA, United States,* [7]*Dept of Neurosciences; University of California, San Diego, San Diego, CA, United States,* [8]*Facultad de Salud Publica y Salud Global; Universidad Peruana Cayetano Heredia, Lima, Peru*

It is known that HIV-associated co-infections may worsen neurocognitive impairment (NCI), but this relationship has not been widely studied in Latin America. As the Peruvian population ages, including Peruvians with HIV, the risk of developing NCI increases. We sought to determine if HIV-associated co-infections worsen NCI among older Peruvians with HIV. We recruited 144 HIV+ Peruvians living in Lima, Peru ≥age 40 from September 2019 to March 2020. Sociodemographic and medical comorbidity (infectious and chronic) data were collected. A brief neuropsychological battery was administered; scores were adjusted for age, sex and education level. NCI was defined as impairment in ≥2 cognitive domains. T-test and logistic regression analyses were performed. Mean age was 52+/-8 years and years of education was 14+/-3; 15% were female. Median (IQR) current CD4 and nadir CD4 was 554 (371, 723) and 179 (83, 291), respectively. Mean time since HIV diagnosis was 10+/- 7 years, 14% had detectable plasma viral load and all were on antiretrovirals. The prevalence of NCI was 20% in the entire cohort and among those with suppressed plasma VL. Past medical history of CNS opportunistic infections (OIs) included toxoplasmosis, herpes encephalitis, cryptococcal meningitis, but CNS OIs were not associated with NCI (p=0.94). About 30% had a positive RPR test, 11% positive Hepatitis B surface antigen (HbsAg) and 8.7% had positive tuberculosis (TB) sputum test in their lifetime; all patients completed treatment. Having a positive lifetime RPR, HbsAg and/or TB sputum test significantly increased odds of NCI (OR 2.8; 95%CI 1.2, 6.4; p=0.015) cumulatively. However, RPR (p=0.09) or TB (p=0.07) positivity separately did not significantly increase odds of NCI, but approached significance. NCI was found to be prevalent among older Peruvians with HIV, and common latent HIV-associated co-infections, including latent syphilis or TB, may increase the risk of NCI among Peruvians. Further studies are required to determine the burden of latent HIV-associated co-infections and their impact on NCI prevalence in Peru.

895

PARVOVIRUS B19 INFECTION IN HIV-INFECTED PATIENTS TREATED AT THE YAOUNDE CENTRAL HOSPITAL CAMEROON

Mauclere Kentsop Ngoula[1], Symphorien Ewodo[2], Charles Kouanfack[3]

[1]*Ecole des Sciences de la Santé, Yaounde, Cameroon,* [2]*Ministère de la Santé Publique/PNTS, Yaounde, Cameroon,* [3]*Faculté de Médecine de l'Université de Dschang, Dschang, Cameroon*

Parvovirus B19 (B19), is a small highly resistant DNA virus responsible for a wide variety of pathologies including cytopenias. Several studies have identified HIV-infected patients, as a particularly vulnerable and high-risk group for this infection. However, the distribution of this virus among HIV-infected patients in Cameroon is not yet known in our resource-constrained context. The aim of this study was to determine the seroprevalence of HIV/B19 co-infection and its possible association with cytopenias. Between July and November 2019, an analytical cross-sectional study including a study group of 60 HIV-infected patients on antiretroviral treatment and a control group of 28 healthy blood donors was conducted at the Yaounde Central Hospital. The prevalence of parvovirus B19 was determined by an enzyme-linked immunosorbent assay (ELISA) detecting B19 IgM and IgG antibodies in the serum of all participants. A blood count and reticulocyte count were also performed on whole blood samples collected in EDTA tubes to look for cytopenias. The prevalence of parvovirus B19 infection in HIV-infected patients and in the control group was 81.67% and 71.43% respectively. B19 IgM antibodies were detected in 30% and 25% of the participants in the study and control groups respectively. Among HIV-infected patients no significant association was found between area of residence, sexe, different categories of HIV viral and B19 IgG antibody. Anemia was the most common cytopenia (38 cases). And among anemic patients, 86.84% (33 cases) were infected with parvovirus B19. 93.93% (31/33) of these parvovirus B19 infected anemic patients had non-regenerative anemias. Under parvovirus B19 infection, the only significant association found was between neutropenia and HIV status but at low risk. These results show us that parvovirus B19 circulates in our environment and the cytopenias observed in HIV-infected patients could be associated with parvovirus B19 infection and could be more severe in cases of deep immunosuppression. Another study on a larger population including AIDS patients would provide a better understanding of its responsibility.

896

HIV PREVALENCE AMONG HIGH RISK MALE AND FEMALE ADULTS SCREENED FOR ENROLMENT IN A VACCINE SITE PREPAREDNESS STUDY IN KISUMU, KENYA

John Owuoth[1], Valentine Sing'oei[1], June Otieno[2], Chiaka Nwoga[3], Adam Yates[3], Ben Andagalu[4], Nathanial K. Copeland[2], Trevor A. Crowell[3], Christina Polyak[3]

[1]*HJF Medical Research International/U.S. Military HIV Research Program, Walter Reed Army Institute of Research, Kisumu, Kenya,* [2]*U.S. Army Medical Research Directorate- Africa, Kisumu, Kenya,* [3]*U.S. Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [4]*U.S. Army Medical Research Directorate – Africa, Nairobi, Kenya*

HIV remains a global public health concern with Kenya reporting high prevalence rates. Strategic focus towards increasing ART coverage remains critical in Kenya's fast track goal towards achieving epidemic control and attaining UNAIDS 95/95/95 targets. The primary goal of this study was to better define HIV incidence and prevalence in male and female adults of high-risk groups in Kisumu, Kenya. Prevalence was based on HIV testing results of male and female participants aged 18-35 screened from high risk groups including men who have sex with men (MSM), female sex workers (FSW) and fisherfolk community. This was done at screening and enrollment visits and HIV behavioral risk assessment questionnaire administered. Of the 1,063 participants screened for HIV infection, 521(49%) were male and 630 (59%) were age 18-26, 204(19%) reported sex worker as their primary occupation and 396 (38%) reported having exchanged money, goods or favors for sex in the past three months. The HIV prevalence was significantly higher in women compared to men (26.4% vs 10.1%, p &lt 0.001), and in participants aged above 30 years old than in younger participants (36.1% vs 13.6%, p&lt 0.001). 25% and 13.5% of participants who reported sex worker and fisherman as their primary occupation were HIV infected. HIV infection was associated with older age 30+ (OR: 4.77, 95%CI: 2.88-7.89), higher level of education seemed protective (OR 0.60, 95%CI: 0.45-0.78). More efforts are required towards reaching populations at high risk of HIV infection with effective HIV prevention strategies and increasing ART coverage.

897

NDARU JAMBO ABSTRACT

Ndaru Jambo[1], Innocent Kadwala[1], Leonard Mvaya[1], Priyanka Patel[1], Joyce Macheso[1], Amber Majidu[1], Anstead Kankwatira[1], Malick Gibani[2], James Meiring[3], Kondwani Jambo[1], Lorena Preciado Llanes[2], Tonney Nyirenda[4], Melita Gordon[5]

[1]*Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre, Malawi,* [2]*MRC Human Immunology Unit, University of Oxford, Oxford, United Kingdom,* [3]*Department of Paediatrics, University of Oxford, Oxford, United Kingdom,* [4]*Department of Pathology, University of Malawi, College of Medicine, Blantyre, Malawi,* [5]*Institute of Veterinary, Infection and Ecological Science, University of Liverpool, Liverpool, United Kingdom*

HIV-infected adults are more susceptible to Invasive Non-Typhoidal Salmonella (iNTS) disease. IFNγ mediated immunity against NTS helps to control intracellular and extracellular spread of the infection. We previously suggested that Gamma Delta (γδ)-T cells are important producers of IFNγ during early *Salmonella* infection. We hypothesized that HIV-infection is associated with impaired γδ-T cell responses against Non-Typhoidal Salmonellae (NTS). Peripheral blood was drawn, and upper gastrointestinal endoscopy performed, on healthy HIV-uninfected, HIV-infected ART-naive, and HIV-infected ART-treated adults. Frequency and functionality of γδ-T cells were assessed using an infection model involving intracellular cytokine staining assay (IFNγ and TNFα) following 18hr stimulation of peripheral blood mononuclear cells (PBMC) and fresh biopsied duodenal tissue cells with an invasive *Salmonella* Typhimurim sequence type 313 (ST313) strain and control stimuli. The frequencies of γδ-T cells were lower in mucosal compared to PBMC samples but were similar in HIV-infected individuals compared to those in HIV-uninfected controls, within both compartments. Considering functionality, significantly lower proportions of γδ-T cells from HIV-infected individuals produced IFNγ and/or TNFα compared to HIV-uninfected individuals. Although individuals on ART showed a slight increase in the proportions of cytokine positive γδ-T cells, pre-HIV-infection numbers were not restored. In conclusion, frequency of γδ-T cells is unaltered during HIV infection in both systemic circulation and duodenal mucosa. However, HIV-infection is associated with impaired functional capacity of γδ-T cells in both compartments and this may contribute to the high susceptibility to and poor clearance of iNTS disease in HIV-infected adults.

898

REPURPOSING OF MEDICINES FOR MALARIA VENTURE'S OPEN ACCESS LIBRARIES LED TO IDENTIFICATION OF POTENT INHIBITORS OF THE CAUSATIVE AGENTS OF SELECTED NEGLECTED TROPICAL DISEASES

Fabrice Fekam Boyom[1], Rufin Marie T. Kouipou[1], Patrick Valere T. Fokou[1], Benoit Laleu[2], Thomas Spangenberg[3]

[1]*University of Yaounde 1, Yaounde, Cameroon,* [2]*Medicines for Malaria Venture, Geneva, Switzerland,* [3]*Merck, Global Health Institute, Eysins, Switzerland*

: F.F. Boyom[1], R.M.K. Toghueo[1], P.V.T. Fokou[1], B. Laleu[2], T. Spangenberg[3]
[1]Antimicrobial & Biocontrol Agents Unit, Faculty of Science, University of Yaoundé 1, P.O. Box 812, Yaoundé, Cameroon, [2]Medicines for Malaria Venture, Route de Pré-Bois 20, CH-1216 Cointrin Geneva, Switzerland; [3]current address: Merck, Global Health Institute, route de Crassier 1, CH-1262 Eysins

Collaboration between the Antimicrobial and Biocontrol Agents Unit (AmBcAU) at the University of Yaoundé 1 and Medicines for Malaria Venture (MMV) over the past seven years to repurpose Open Access libraries of compounds including the Malaria Box, Pathogen Box and Stasis Box using standard laboratory methods has identified potent inhibitors of *Mycobacterium ulcerans*, *Entamoeba histolytica* and *Toxoplasma gondi* that cause two NTDs (Buruli ulcer, amoebiasis) and toxoplasmosis respectively. Overall, phenotypic screening of compounds libraries and subsequent structure-activity-relationship studies revealed novel scaffolds with low micromolar to nanomolar range activity against *Entamoeba histolytica* (MMV1578523-IC_{50} = 43 nM; MMV1578540-IC_{50} = 55 nM), *Mycobacterium ulcerans* (MMV1578876- IC_{50} = 0.52μM; MMV1578877- IC_{50} = 0.37μM), and *Toxoplasma gondi* (MMV007791-IC_{50} = 0.19 μM; MMV007881-IC_{50} =1.07 μM; MMV007363-IC_{50} = 1.49 μM; MMV006704-IC_{50} = 1.95 μM; Z356179656- IC_{50} = 0.13 μM). Further, PK studies indicated high rate of degradation for the anti-*M. ulcerans* hit (MMV1578877) in liver microsomes and high solubility, acceptable stability in human microsomes, but relatively high metabolic clearance in rat hepatocytes for the two anti-*Entamoeba* hits. Hit-to-lead studies are ongoing.

899

IMPACT OF DIFFERENT INTERVENTION STRATEGIES ON HELMINTH AND INTESTINAL PROTOZOA, IN CENTRAL CÔTE D'IVOIRE

Gaoussou Coulibaly

University Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire, Abidjan, Côte D'Ivoire

Intestinal parasitic infections due to helminths and protozoa are a major public health problem in developing countries. They are one of the main causes of childhood morbidity and mortality worldwide. Preventive chemotherapy is the strategy to control helminthiases. However, rapid reinfection occurs in settings where there is a lack of clean water, sanitation and hygiene. In order to better understand the impact of an integrated control approach, combining preventive chemotherapy, sanitation and health education, on helminth and intestinal protozoa infections, a cluster randomized trial was conducted in 54 localities of three (3) sub-prefectures, in central Côte d'Ivoire. Study participants were invited to provide stool and urine samples, was examined according to standard diagnostic methods. A questionnaire was administered to households to collect data on socio-demographic characteristics, sanitation and hygiene practices. Hookworm was the predominant helminth with an overall prevalence of 21.2 %. This parasite was associated with open defecation. Pathogenic intestinal protozoa were found at similar prevalences, following localities. Poor hygienic conditions were correlated with *Giardia lamblia*. Interventions focused on preventive chemotherapy coupled with community-led total sanitation or the community health education program have significantly decreased, prevalence and infection intensities of *Ankylostoma sp*., more than 90 %. Concerning intestinal protozoa, the highest reduction rates of *Entamoeba histolytica* (66 %) and *G. lamblia* (80 %) prevalence were observed in the groups receiving preventive chemotherapy and community health education program. Such results are important for national control programmes targeting neglected tropical diseases to the development of an integrated control program.

900

INTEGRATED ALTERNATIVE COMPLEMENTARY TREATMENT STRATEGIES (ATS) TO ACCELERATE RIVER BLINDNESS ELIMINATION IN CAMEROON

André Domche

University of Yaoundé I, Yaoundé, Cameroon

The control of onchocerciasis relies essentially on mass drug administration (MDA) through the so-called Community-Directed Treatment with Ivermectin (CDTI) strategy. Although CDTI had helped to reduce/interrupt transmission elsewhere in Africa, the disease is persisting in some foci in Cameroon despite more than 30 years MDA. This suggests a need of alternative control strategies. This study therefore aims to accelerate the momentum towards onchocerciasis elimination using alternative and/or complementary strategies to CDTI. This interventional study will be organized in two arms in the Centre Region of Cameroon, one implementation (Biatsota village) and one control (Bayomen village). In each arm, the population dynamics of blackflies will be assessed over a year. Interventions (blackfly trapping and physical destruction (slash and clear) of their breeding sites, and optimal timing of CDTI) will be carried

out during the next two years at three-monthly interval. During the third year, blackflies will be collected both in the interventional and control sites, to assess the impact of intervention on the blackflies' densities. River blindness exposition will be measured among children under 5 years old, prior- and post- interventions, using the Ov16 RDT. We are expecting that these integrated interventions will lead in significant reductions in blackfly densities, and consequently a significant reduction in onchocerciasis prevalence, intensity of infection and transmission. The study is ongoing but at 6 months after initiation, a total of 32,969 (16,907 in Bayomen and 16,062 in Biatsota) blackflies have already been captured. A total of 18,253 (%) have been dissected for parity, the remaining being stored for morphological identification and pool screening. The highest mean daily biting rates being observed in November, at the end of the long rainy season suggesting a seasonal variation in blackfly densities. A total of 920 (5.04%) dissected flies were parous, 28 (3%) of them harbouring *Onchocerca* spp larvae. The highest monthly transmission potentials were also observed in November.

901

ASSESSING THE EXTENT OF COVERAGE AND COMPLIANCE TO IVERMECTIN MASS DRUG ADMINISTRATION AND ASSOCIATED FACTORS AMONG RESIDENTS IN ONCHOCERCIASIS ENDEMIC COMMUNITIES IN OWABI CATCHMENT AREA IN GHANA

Francis Adjei Osei[1], Ellis Owusu-Dabo[1], Sam K. Newton[1], Nicholas Karikari Mensah[2], Evans Xorse Amuzu[2], Samuel Frimpong Odoom[2], Isaac Nyanor[2], Suraj Yawnumah Abubakar[2], Bright Atta Dankwah[2], Ebenezer Opoku[1], Alfred K. Owusu[1], Phans Oduro Sarpong[3], Stephanie Boadi[2], Ernest Amanor[2], Peter Furu[4], Dan Wolf Meyrowitsch[4]

[1]*School of Public Health, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana,* [2]*Komfo Anokye Teaching Hospital, Kumasi, Ghana,* [3]*Kumasi Centre for Collaborative Research KNUST, Kumasi, Ghana,* [4]*Department of Public Health, Global Health Section, University of Copenhagen, Copenhagen, Denmark*

Mass Drug Administration (MDA) of Ivermectin has proven to be safe and potent microfilaricide against *Onchocerca volvulus*. In Africa, coverage and compliance rates mostly fall below the World Health Organisation (WHO) target of 80% and 65% respectively. The study examined Ivermectin coverage, compliance and its enabling factors from the community inhabitants' perspective. We conducted a cross-sectional study in four randomly selected communities (Dabaa, Owabi, Koforidua and Ntensere) in the Ashanti region of Ghana where MDA of Ivermectin has been ongoing for two decades. The 2,008 participants were randomly sampled and recruited for the study based on the eligibility criteria. Data was collected using REDCap Mobile App and analysed using STATA 16.0. Multiple logistic regression model was used to identify factors associated with coverage and compliance at 5% significance level. The median age of the participants was 30 years (IQR=22-42 years). A significant proportion of the respondents (63.94%, n=1,284) did not receive Ivermectin drug during the last MDA. Reasons given for non-receipt included unawareness of the drug distribution (53.27%, n=684) and absence from home (25.23%, n=324) during the distribution. Out of the 724 (36.06%) of the respondents who received the drug, compliance (persons who swallowed the drugs) was high, 91.02% (n=659). Compliance was significantly associated with perceived benefit of Ivermectin (AOR 4.1, 95% CI=1.685-10.147), awareness of MDA program (AOR- 2.3, 95% CI=1.003-50186) and participation in previous MDA campaigns (AOR= 10.7(5.589-20.384). MDA coverage was low in the study communities. The reasons given by respondents who did not receive the drugs gives an indication of implementation challenges. An improved social mobilisation effort which targets persons living at peri-urban areas should be explored. There is also the need to improve awareness and education on MDA in the study areas for enhanced systematic compliance.

902

NEED OF HEALTH POLICY AND SYSTEM RESEARCH FOR INTEGRATED CONTROL OF NEGLECTED TROPICAL DISEASE

Sehrish Ather[1], Zakria Tariq[2], Romana Ayub[3]

[1]*University of Lahore, Lahore, Pakistan,* [2]*Allama Iqbal Medical college Jinnah Hospital, Lahore, Pakistan,* [3]*Khyber Medical College, Peshawer, Pakistan*

To improve organizational achievements through the production of new knowledge health policy and system research is the basic requirement. An increased number of neglected tropical diseases in countries is always the source of increased burden of diseases, disabilities and other co-morbidities; therefore provision of integrated approach to control the neglected tropical diseases in every country should be achieved by making strong policy and system research for the betterment of health care system and prevention of increased burden of diseases. Unfortunately, the whole world is lacking strong policies and system research for controlling the neglected tropical diseases. A literature review of published studies on neglected Tropical diseases was done, ranging from year 2011-2019. Databases searched were Google Scholar, PubMed, Science Direct, Ovid and Research Gate. Grey literature was searched from various websites including Institute for Health Metrics and Evaluation, World Health Organization and Personal communication with CDC Team management. After careful reviewing of published and un-published information it was decided to carry on with commentary. Most of the published studies have highlighted the need to advocate the funders of health policy and stakeholders of healthcare system research and it was detected as major issue, research on policy and healthcare system to prevent and control neglected tropical diseases was found as highly neglected area. It is concluded that scientists and researchers of basic and social sciences are less likely to be involved in methods used for health policy and system research due to the lack of funding and resources. Therefore neglected tropical diseases should be considered as priority and comprehensive policy and system research should be initiated for these diseases as most of these diseases share the same geographical and epidemiological distribution so the integrated approach to control and prevent these diseases will work more effectively if proper policy making and system research will be advocated. 1

903

PERSPECTIVES AND CONCERNS OF IMPLEMENTERS ON INTEGRATED CONTROL OF NEGLECTED TROPICAL DISEASES INTO PRIMARY HEALTH CARE IN NIGERIA

Chinenye Afonne[1], Tony Danso-Appiah[1], IkeOluwapo Ajayi[2], Patricia Akweongo[1], Patricia Nortey[1], Solomon Offor[3], Bertram E. Nwoke[4], Catherine O. Falade[2]

[1]*University of Ghana, Legon, Ghana,* [2]*University of Ibadan, Ibadan, Nigeria,* [3]*State Ministry of Health, Umuahia, Abia State, Nigeria,* [4]*Imo State University, Owerri, Nigeria*

Integrating Neglected Tropical Diseases (NTDs) programs into primary healthcare has been suggested as the ideal and sustainable strategy for achieving equity through universal health coverage, in sub-Saharan Africa. However, the perspectives of implementers are very significant and if well studied would proffer evidence-based information for planning and scale-up. We present results of qualitative research from Abia State in South-Eastern Nigeria, where integrated preventive chemotherapy for NTDs commenced in 2014. Interviews were conducted with Program managers, Donor representatives and Data Management staff at State and Local government area (LGA) levels, about their views on the subject. A total of 22 interview transcripts were transcribed and reviewed for content analysis. Several issues for consideration emerged. Implementers expressed concerns that integrating NTD programs into the existing national health system is a complicated and demanding feat at present. Donor representatives and Program managers affirmed that not all aspects of different health programs could be integrated due to differences in target populations, delivery strategies and data collection instruments.

The LGA level implementers believe they could co-implement with other health programs on community engagement, sensitization and awareness, deployment of community drug distributors in the event of outbreaks, immunization and other emergency health issues. For data management, integrated systems for case-finding, mapping, surveillance, data collection and reporting of operational health programs including NTDs could be designed and streamlined. Divergent interest and focus of donor agencies, information hoarding, lack of locally generated funds were key challenges to integration. The willingness of donors to adjust, understand what to integrate and what not to integrate, and availability of funds were other concerns highlighted. A central and accessible surveillance system, integrated proposals, regular deliberations and coordinated planning by donors, basket funding enforced by Federal Ministry of Health were recommended.

904

CLOSING THE NEGLECTED TROPICAL DISEASES CROSS SECTOR COORDINATION GAP: AN ORGANIZATIONAL NETWORK ANALYSIS TO SUSTAIN ELIMINATION AND CONTROL OBJECTIVES IN GHANA

Arielle Dolegui, Stephen O. Omunyidde

World Vision, Washington, DC, United States

As Ghana progress towards achieving elimination and control objectives for Neglected Tropical Diseases (NTDs), there is a pressing need for tailored cross sector coordination interventions to sustain the MDA gains . To this end, a baseline analysis of current stakeholder networks is critical to identify current gaps in collaboration. We conducted organizational network analyses (ONA) to illustrate current coordination networks among partners and identify existing gaps in NTDP collaboration and engagement with other key sectors or programs such as education, nutrition, malaria, WASH, maternal and child health and agriculture. Collaboration was determined by four factors: communication between actors, degree of information sharing, shared decision making, and shared planning and program implementation. 41 key informant interviews were conducted. The respondents named and rated their interactions with their key NTD partners on four factors with a scale from 1 (low) to 5(high) The aggregate score provided a proxy score that represents the level of collaboration between actors. Kumu.io software was used to analyze the connectedness and collaboration of partners. The parameters for centrality and connectivity were degree centrality (number of connections for a partner), closeness centrality (distance each partner is from all other partners), and betweenness centrality (the flow of information between partners). The resulting network of NTD actors showed a strong divide between the NTD and WASH partners. The NTDP is relatively isolated within the network—neither bridging across health programs, nor across external sectors. While there are some programs that link the NTD partners across areas—notably the School Health Education Program (SHEP), and the Health Promotion Unit—generally, NTD partners are siloed. This also holds true for connectivity between NTDP and malaria, nutrition, and maternal and child health partners. These quantitative ONAs illustrating collaboration gaps provide a baseline for targeted cross sector interventions to protect current NTDP investments and promote a path towards sustainability.

905

KNOWLEDGE CONCERNING ZOONOSES AMONG MEDICAL PRACTITIONERS IN LUSAKA, ZAMBIA

Gelly S. Chikuni[1], Esraa M. Mostafa[2], Le Huu Nhat Minh[3], Ahmed M. Sayed[4], Gehad M. Tawfik[5], Jens Byskov[6], Nguyen Tien Huy[7], Kenji Hirayama[8]

[1]*Ministry of Fisheries and Livestock, Luwingu, Zambia,* [2]*Faculty of Medicine, Port Said University, Port Said, Egypt,* [3]*Faculty of Medicine, University of Medicine and Pharmacy, Ho Chi Minh City, Viet Nam,* [4]*Faculty of Pharmacy, Al-azhar University, Cairo, Egypt,* [5]*Faculty of Medicine, Ain Shams University, Cairo, Egypt,* [6]*Emeritus Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark,* [7]*School of Tropical Medicine and Global Health, Nagasaki University, Nagasaki, Japan,* [8]*Department of Immunogenetics, Institute of Tropical Medicine (NEKKEN), Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan*

Zoonoses cause about 60% of communicable diseases and are the reason for 75% of emerging human pathogens. We investigated the knowledge of zoonoses among medical practitioners as an indication for their capability to perform zoonoses diagnosis, treatment and prevention. The study design employed was cross-sectional with the questionnaire as the tool for data collection.151 medical practitioners were recruited from the Children's and Adult Hospitals of the University Teaching Hospital in Lusaka, Zambia. To evaluate the responses to each question, a score of 2 was given for correct, 1 for partially correct, and 0 for incorrect. The questionnaire had five knowledge domains namely 1) general concepts, 2) causes, 3) mode of transmission, 4) clinical signs and 5) specific zoonotic diseases (rabies and anthrax), with each domain including 7, 4, 6, 6, and 8 questions respectively.The results showed that medical practitioners from the infectious disease department were aware with zoonoses more than their colleagues in internal medicine, general practitioners, and general surgery. Knowledge level was associated with older age (B-coefficient = 0.237, 95% CI [0.08-0.394]; P= 0.003) and duration of service (B-coefficient = 0.206, 95% CI [0.046-0.366]; P= 0.012). After adjusting by multi-level linear regression; the results showed that self-study caused a surge in zoonoses knowledge (B-coefficient = 0.300, 95% CI [0.033-0.566]; P= 0.028), more than practical experience (B-coefficient = 0.201, 95% CI [0.022-0.380]; P= 0.028), and study during the bachelor degree (B-coefficient = 0.202, 95% CI [0.032-0.373]; P= 0.020).This study has revealed a shortage of knowledge about zoonoses among medical practitioners at the Children's and Adult Hospitals of the University Teaching Hospitals in Lusaka, Zambia. Unexpectedly self-studying medical practitioners had more knowledge than those with practical experience or with higher education. It is therefore recommended that self-studying tools be implemented in order to cover the gap of zoonoses knowledge.

906

PREDOMINANCE OF *RICKETTSIA AFRICAE,* THE ETIOLOGIC AGENT OF SPOTTED FEVER GROUP RICKETTSIAE IN TICKS COLLECTED FROM DOMESTIC ANIMALS IN RAYMOND NKANDLA MUNICIPALITY, EASTERN CAPE, SOUTH AFRICA

Benson Chuks Iweriebor [1*]; Larry Chikwelu Obi [1] 1. Sefako Makgatho Health Sciences University, Ga-Rankuwa, Pretoria, South AfricaCorrespondence should be addressed to Benson C Iweriebor benvida2004@yahoo.com

Ticks have the propensity of transmitting a plethora of pathogens that have zoonotic potential. The distribution and diversity of ticks and the pathogens they transmit differs from one ecological location to another. SFG rickettsiae which are predominantly transmitted by different Ixodid ticks are responsible for emerging zoonotic diseases globally. Ticks were collected from domesticated animals in Raymond Nkandla Municipality, Eastern Cape, South Africa. They were identified morphologically prior to processing for DNA extraction. The extracted DNA samples was used to molecularly identify some randomly selected ticks as well as assess for the presence of tick-borne pathogens belonging to *Rickettsia* spp. by PCR using specific primer pairs targeting the *glt*A, *omp*A and *omp*B. All amplified ticks, positive *omp*B and forty three *omp*A amplicons were sequenced in a commercial sequencing facility. The obtained nucleotide sequences were subjected to BLASTn for homology search and phylogenetic analyses performed with MEGA 7 Version for evolutionary relationships with curated reference sequences in GenBank. A total of 953 ticks collected in the study were morphologically and molecularly delineated into three genera consisting of *Amblyomma, Rhipicephalus* and *Hyalomma* in decreasing order of abundance. The presence of *Rickettsia* DNA was detected in 60/953 (6.3%) from the three genera of ticks screened. Evolutionary genetic analyses of the sequences obtained showed phylogenetic relationship to members of spotted fever group rickettsiae with *R. parkeri* and *R.tamurae* along with *R africae* being the SFGR

detected in the ticks screened. This is the first report on genetic evidence of *R.parkeri* and *R.tamurae* in ticks collected in the African continent and the human health impacts are not known.Key words:- Ticks, South Africa, spotted fever group rickettsia, *R. parkeri, R. tamurae, R. africae,* zoonotic potentials.

907

ARE BAT HUNTERS AWARE REGARDING BAT-BORNE DISEASES? A QUALITATIVE STUDY AMONG A BAT-HUNTING COMMUNITY IN BANGLADESH

A.K.M. Dawlat Khan

Department of Anthropology, University of Dhaka, Dhaka, Bangladesh

Bat harbors many infectious agents like Nipah virus (NiV) that spillover from bat to human through the date palm sap, partially eaten fruits or contaminants of bat's excreta and secretions. Since 2001 in Bangladesh, NiV human outbreaks have been occurring with a more 70% case fatality. Consumption and selling bush-meat can cause serious zoonotic diseases like COVID-19 and Ebola. However, bat hunting is a common practice in several parts of Bangladesh due to its local delicacy and medicinal properties. To conceptualize their understanding of bat-borne diseases during May 2017-April 2018, I have collected data from the 60 households through structure questionnaire and in-depth interviews with 4 bat-hunters, and 20 persons who were engaged in butchering, processing, and selling of bat. They work as day laborers in jute mills and storehouses during the jute harvesting time from July to September, but in winter the people were in lack of work and many of them hunt bats and sell meat without any personal protection. This bat slaughtering is placed at their homestead that also could lead the risk of infections to family members and other animals as carcasses are not properly buried. I found most of the household heads and other bat hunting people did not believe that any disease can transmit from the bat. This is because they expressed that bats are clean as they are flying top of the tree and there is no dirt or diseases on there. In Bangladesh, no direct evidence of laboratory confirmed NiV cases found among bat hunters. But, one hunter in the study village had developed Nipah like encephalitis syndromes and died before testing and the majority believed that it was happened due to some supernatural causes. WHO listed NiV is a 'Blueprint priority disease in 2017. Campaign against NiV mostly posters and pamphlets regarding discouragement of consumption of raw date palm sap and partially eaten fruit and grossly ignored bat-meat consumption. As no vaccine or effective drugs are discovered against NiV, we need to adopt a culture-sensitive intervention with the economic outcomes of these hunters to reduce the upcoming threat of zoonotic diseases.

908

EXPERIENCE IN IMPLEMENTING THE "ONE HEALTH" APPROACH IN INTEGRATED CONTROL OF EMERGING DISEASES IN GUINEA

Alpha Ahmadou Diallo

Ministry of Health and University of Conakry, Conakry, Guinea

Guinea was affected by the Ebola, the magnitude of which increased inequities in access to health care. In the post-Ebola context, a capacity assessment was undertaken in relation to the International Health Regulations. The scope of this strategic direction is to assess the country's capacity to prevent the spread of infectious diseases & the appropriate response measures to deal with them: detecting, reporting and controlling these diseases & mitigating the impact of public health threats through combined health action. Assess the country's capacity to ensure surveillance of diseases with epidemic potential, preparedness & adequate response through the implementation of the One Health approach. This is a descriptive study with an analytical focus. It was conducted in three health districts in Guinea. A total of 378 key informants. Most (96%) have heard about emerging diseases including Ebola, dengue fever, monkey pox through the media &/or education. Only 39% adopt good safety practices in the community. In health care settings, all providers adhere to universal biosafety precautions. Nearly half of health professionals (47%) rated the potential risk of an epidemic as high. While the authorities' effort is described as weak in 42% of cases, as well as community involvement, the contribution of partners is strong in 66%. In conclusion, strategic and operational aspects such as the need for strong political commitment, multisectoral collaboration - stakeholders interacting continuously for the same purpose and a monitoring and evaluation framework to inform progress and gaps in real time were jointly addressed.

909

EFFECTS OF ROAD NETWORK AND POPULATION DENSITY ON THE RISK OF RABIES AND DOG BITE INCIDENCE IN NIGERIA

Christianah C. Odita[1], Ishaya S. Tekki[1], Abass A. Adigun[2], Israel I. Barde[1], Emmanuel S. Hambolu[1], Moses G. Gyang[1], Joseph D. Alex[1], Dashe Y. Yakubu[1], Chika C. Nwosuh[1]

[1]*National Veterinary Research Institute Vom Plateau State Nigeria, Jos, Nigeria,* [2]*National Centre for Remote sensing Jos Plateau State Nigeria, Jos, Nigeria*

Canine rabies is endemic in Nigeria and associated with high rates of human exposure. Although efforts by stake holders at different times involving mass vaccination of dogs have been made, the disease occurrence continue to persist among domestic dogs responsible for transmission to humans and other domestic animals. To make progress in the elimination of the disease, factors associated with its continued persistence must be identified and addressed. This study investigates whether a location's road network and population density are associated with the risk of having high prevalence of dog bite incidents. Exploratory spatial analysis in addition to cross sectional study of records of dog bite incidents (2015-2019) of the National Veterinary Research Institute, Vom was conducted. A total number of 577 cases involving 17 states, Federal capital territory, and 55 administrative areas of Nigeria with complete information on locations and victims were isolated. Incident locations (states) were split into two (greater than 10 cases, and less than 10 cases of dog bite incidents), and whether they (administrative areas) have standard roadways passing through them or not. Data analysis indicates that incident locations with greater than 10 cases have more standard roadways (odds ratio 4.9; 98% CI 1.7 - 14.9); and possess higher population density (average 1489.76 per 100 square metre against 206.39 per 100 square metre). Dog bite prevalence is 12.3 times higher in communities with more standard roadways. Average Normalized Difference Vegetation Index is similar in both communities. Although we cannot conclude a causal link, there is evidence of statistical relationship between increased number of standard roadways and prevalence of dog bite and hence rabies. Standard roadways may play a crucial role in the persistence of rabies primarily due to ease of transportation, and high movement of people and animals in communities where they pass through. The study aims to target surveillance and control measures on specific at risk communities towards eliminating rabies in domestic animals, and dog bite-associated rabies incidences in humans.

910

ENVIRONMENTAL CHANGES AND POTENTIAL BAT-BORNE JAPANESE ENCEPHALITIS VIRUS TRANSMISSION IN BALI, INDONESIA

Ajib Diptyanusa, Tri Baskoro T. Satoto, Elisabeth S. Herini, Soedarmanto Indarjulianto

Universitas Gadjah Mada, Yogyakarta, Indonesia

The Bali province in Indonesia belongs to endemic area of Japanese encephalitis (JE) that accounted for approximately 42% of total cases yearly, even after the commencement of JE vaccination campaign in 2017. Interestingly, the clinical cases of JE were mostly found residential areas not in close proximity to any pig farms, hence the possibility of another reservoir host that might have played a role in the transmission of JE virus in Bali, Indonesia. Deforestation, rapid urbanization and evolving land-use

in Bali might have led to setting up of bat population in areas closer to human dwellings, increasing the risk of bat-borne virus transmission. Current study aimed to identify the potential role of bats (Chiroptera) in the local transmission of JE by performing molecular detection of JEV in bat serum using RT-PCR. Sample collection was performed in 3 districts in Bali, covering 3 different ecosystems: woodland, coastal, and residential areas. Bat collection was performed using mist net and harp net. A total of 356 bats were captured and were included in the study, among which the majority belonged to genus *Cynopterus* (52.0%), followed by genus *Rousettus* (16.0%) that were all captured in non-forested areas. The RT-PCR testing for JEV showed positive result from 1 sample taken from *Cynopterus brachyotis* (lesser short-nosed fruit bat) found in residential areas. While most serum samples showed negative results, it is imperative to note that many of the bats were not captured in their natural habitats. Current study results demonstrated the urgent need of aggressive One Health measures to address the potential role of bats in local transmission of JE in Bali through averting disruption of bats' natural habitats by human actions.

911

FELINE-HUMAN ZOONOSES TRANSMISSION IN NORTH AFRICA: A SYSTEMATIC REVIEW

Breck N. Peterson, Amber N. Barnes

University of North Florida, Jacksonville, FL, United States

Throughout human history, domestic animal species have represented a unique zoonotic disease risk for the transmission of pathogens ranging from viral, bacterial, parasitic, and fungal. In North Africa, cats have a particularly long record and occupy a specialized niche within many communities. This systematic review was conducted to analyze the current and historical literature documenting the breadth and variety of zoonoses in North Africa, specifically relating to the domesticated feline. Multiple electronic databases were searched on January 16, 2019 for published reports on feline zoonoses in North Africa. A total of 76 studies met the inclusion criteria for a full assessment. Articles selected for the review ranged in publication dates from 1939 to 2019 and included a case study, cross-sectional surveys, genomic analyses, and a book chapter. The most commonly studied pathogen was Toxoplasma gondii (n=17) followed by a variety of helminths (n=10). Of the countries in the target region, most publications were of studies conducted in Egypt (n=53) followed by Tunisia (n=12), Algeria (n=11), Morocco (n=5), and Libya (n=3). The results of this review identify a variety of viral, bacterial, fungal, and parasitic zoonotic diseases associated with cats in North Africa, ranging from historically endemic diseases in both human and animal populations in the region, to emerging infections with recent confirmatory diagnoses. This review describes reported feline zoonoses in North Africa and provides recommendations for their prevention and control. In addition to vaccination campaigns for domesticated felines and post-exposure prophylaxis for humans, prompt veterinary and medical care of exposure risks and subsequent infections is essential in limiting the zoonotic disease burden in North African communities of humans and cats.

912

MAPPING THE ENVIRONMENTAL SUITABILITY OF MONKEYPOX IN HUMANS ACROSS AFRICA

Erin N. Hulland[1], Austin N. Hardcastle[1], Joshua C. Osborne[1], Julia D. Morgan[1], Shreya Shirude[1], Kiana F. Henny[1], Peter Rabinowitz[2], Judith N. Wasserheit[2], Molly K. Miller-Petrie[1], Julia Hon[1], Simon I. Hay[1], David M. Pigott[1]

[1]*Institute for Health Metrics and Evaluation, Seattle, WA, United States,* [2]*University of Washington School of Medicine, Seattle, WA, United States*

Monkeypox virus (MPXV) is a re-emerging zoonotic disease, dubbed the most important orthopoxvirus in humans since the eradication of smallpox in 1977. Largely hidden during the smallpox era due to similarity of symptoms and cross-immunity of smallpox vaccination, MPXV was first found in humans in 1970 in a 9-month-old boy from the Equateur province of the Democratic Republic of Congo (DRC) and has since been the cause of several outbreaks in humans across central and west Africa, extending as far as the United States in 2003 and more recently into the United Kingdom, Singapore, and Israel. Although MPXV was first extracted in 1958 from a cynomolgus monkey, investigations into the reservoir of MPVX have suggested primates are not the natural reservoir and instead point to small mammals like rope squirrels and pouched rats, abundant across Africa. Given the uncertainty around the reservoir, analytic methods are essential for estimating where MPXV transmission may occur based on environmental signals and case reports. In this analysis, we use openly available data from published literature and epidemic case reports of MPXV in humans and animals and combine these data with geospatially resolved climatic and landcover data at a monthly resolution. We use boosted regression tree models to estimate the suitability for MPXV transmission on a monthly basis at a 5km-by-5km resolution and demonstrate where the probability of spillover into human populations is highest based on suitability and population density. Our models suggest suitability of MPXV transmission for all months of the year across central and west Africa, both in places where the disease has been known to occur like in DRC, but also in novel locations, such as Guinea in west Africa and Angola in central Africa. Recent events have demonstrated the need for pandemic preparedness more than ever, and spatially and temporally resolved maps are an invaluable tool for prioritizing and allocating resources and efforts. Our analyses demonstrate where MPXV transmission is most likely to occur and the magnitude of the population affected, providing much needed evidence for MPXV preparedness activities.

913

PERINATAL EXPOSURE TO BISPHENOL A INCREASES IN THE ADULTHOOD OF THE OFFSPRING THE SUSCEPTIBILITY TO THE HUMAN PARASITE *TOXOCARA CANIS*

Victor H. Del Rio-Araiza, Karen E. Nava-Castro, **Jorge Morales-Montor**

Universidad Nacional Autónoma de México, Instituto de Investigaciones Biomedicas, Ciudad Universitaria, Mexico

Bisphenol A, a very widespread environmental pollutant and endocrine disruptor compound, can interact with several steroid receptors, particularly with estrogen ones. In different studies, it has observed that the endocrine disruption during critical periods of development can trigger alterations in the immune response during the adult life. Male Wistar rats were exposed indirectly to BPA at a dose of 250µg/kg day during the perinatal period (from day 5 of pregnancy until day 21 postnatal), At the 60 days of age, the adulthood, animals were infected with larvated eggs of the *Toxocara canis*, and were sacrificed at 7 days post-infection. Parasitic loads in the lung and in the liver were analyzed by artificial digestion. Furthermore, immune cell subpopulations (macrophages, NK cells, Tγδ, total T cells, T helper, T cytotoxic, and B lymphocytes) present in spleen, peripheral and mesenteric lymph nodes were analyzed by flow cytometry. The expression of Th1 and Th2 cytokines at the splenic level was determined by real-time quantitative PCR. Finally, the titers of specific antibodies against to the parasite were analyzed by ELISA. The BPA treatment administrated in the perinatally stage favors a significant increase of the percentage of *Toxocara canis* larvae in the lungs and liver in the adulthood. Additionally, the exposure to this compound caused a dramatically decrease in the production of specific antibodies against to this parasite, downregulating together Th2 cytokines (IL-4, IL-5 and IL-13), meanwhile upregulated Th1 cytokines (IFN-γ and TNF-α). Perinatal exposure to BPA affects the performance of the immune response during adult life, modifying both cytokines and antibodies production by these cells, which favors the susceptibility to infections, specifically toxocariosis.

914

ONE HEALTH APPROACH IN RABIES MANAGEMENT IN SARAWAK, MALAYSIA: WHERE ARE WE?

Nur Asheila Abdul Taib, Jane Labadin, Phang Piau

Universiti Malaysia Sarawak, Kota Samarahan, Malaysia

The previously rabies-free Sarawak, which is an East Malaysian state located in the Island of Borneo, has been battling with the ongoing rabies epidemic ever since its first outbreak in July 2017. As of March 2020, there had been 22 cases of human rabies deaths in which 100% of the cases were canine-mediated. Although the outbreak now is in its fourth year, the rate of bite incidence from dogs and cats is still relatively high and similar to that of the first year. Our work aimed to rationalize the One Health concept by investigating the effectiveness of various rabies control measures in Sarawak using a deterministic epidemiological model that includes dog and human populations. We also incorporated the public education efficacy in regard to dog roaming habits, dog containment and the practice of responsible pet ownership into our model. We have fit our model to the actual monthly prevalence data concerning human rabies in Sarawak from June 2017 to March 2020 and analysed the transmission dynamics of Sarawak rabies. Numerical simulation depicted low-level endemic rabies transmission in Sarawak for at least 8 years. The basic reproduction number is estimated to be 3.07 which indicates high rabies prevalence within the canine population in Sarawak. We performed sensitivity analysis of the basic reproduction number in terms of model parameters to identify the influential factors for rabies eradication in Sarawak. The findings implied the need to strengthen the current rabies control efforts by increasing the rate of dog vaccination, rate of human vaccination and public education intervention as well as reducing the number of newborn dogs in Sarawak. The results also suggest that the collaborative One Health approach between the health department, veterinary department and the city council should be intensified in order to achieve the goal of zero canine-mediated human rabies death by the year 2030.

915

INFECTIOUS DISEASE AND VECTOR SURVEILLANCE IN WEST AFRICA

Naiki Puplampu[1], Shirley Nimo-Paintsil[1], Anne T. Fox[1], James Harwood[2], Matthew Montgomery[3], Joseph Diclaro[3], William K. Ampofo[4], Samuel Dadzie[4], Kennedy K. Addo[4], Andrew Letizia[5], David Wolfe[1], **Chase Watters**[1], Terrel Sanders[1]

[1]*Naval Medical Research Unit-3-Ghana, Accra, Ghana,* [2]*Naval Medical Research Unit-3, Sigonella, Italy,* [3]*Navy Entomology Center of Excellence, Jackson, FL, United States,* [4]*Noguchi Memorial Institute for Medical Research, Accra, Ghana,* [5]*Naval Medical Research Center, Silver Spring, MD, United States*

Emerging infectious diseases pose significant threats to service members in the field and ultimately global security. Ongoing and previous disease outbreaks, such as COVID-19, 2018-2020 Ebola in the DRC, and 2014-2016 Ebola in West Africa, emphasize the need for rapid field detection and response capabilities in remote regions. The mission of Navy Medical Research Unit-3 (NAMRU-3) is to ensure warfighter readiness by detecting, deterring, and responding to infectious disease threats through in-country collaborations. Partnering with the host country to accomplish this mission is critical, as it is more effective, eliminates operating challenges in remote regions, promotes global security, and generates expertise to deal with future disease outbreaks. To accomplish its mission NAMRU-3 partners with other DoD institutes, Noguchi Memorial Institute for Medical Research - University of Ghana, Ghana Armed Forces, and the Ghana Health Service, enabling sampling at multiple field sites throughout Ghana, Togo, Cameroon, Liberia and Burkina Faso. Specifically, this has led to surveillance of chemoprophylaxis-resistance of malaria causing-*Plasmodium falciparum* from 2007-2016, antibiotic resistance of STI-causing *Neisseria gonorrhoeae* from 2012-2015, and weekly flu rates from 2007-2020. Surveillance of vector-borne diseases (ticks and mosquitoes) in five regions of Ghana began in 2016 and is currently ongoing. In Cameroon, surveillance of dengue-vector mosquitoes (2019-2020) determined mosquito species distribution (*Aedes aegypti* and *Aedes albopictus*) in highly populated areas. Most recently, NAMRU-3's in-country partnerships enabled rapid detection of COVID-19 in local patient samples. Advanced diagnostics used for surveillance include RT-PCR, next generation sequencing, and immunological assays. Overall, these data provide commands with disease profiles of concern for deployed troops, and epidemic response capabilities. Recent outbreaks highlight the importance of continued surveillance to detect and neutralize future threats.

916

COSTA RICA WILDLIFE REPORTING OF DISEASE DATA FOR PREVENTIVE MEDICINE AND DISEASE CONTROL

Tamara Danielle Solorzano[1], Fernando Aguilar[2], Ana Jiménez[3], Elías Barquero[4], Martha Piche[5], Gaby Dolz[6], Carlos Jiménez[5], Mario Baldi[7], Bernal León[8], Mario Santoro[9], Alejandro Alfaro[1]

[1]*Departamento de Patología, Escuela de Medicina Veterinaria, Universidad Nacional, Costa Rica, Heredia, Costa Rica,* [2]*Programa de Vida Silvestre, Servicio Nacional de Salud Animal, Ministerio de Agricultura y Ganadería, Costa Rica, Heredia, Costa Rica,* [3]*Departamento de Parasitología, Escuela de Medicina Veterinaria, Universidad Nacional, Costa Rica., Heredia, Costa Rica,* [4]*Departamento de Bacteriología, Escuela de Medicina Veterinaria, Universidad Nacional, Costa Rica., Heredia, Costa Rica,* [5]*Departamento de Virología, Escuela de Medicina Veterinaria, Universidad Nacional, Costa Rica., Heredia, Costa Rica,* [6]*Departamento de Zoonosis, Escuela de Medicina Veterinaria, Universidad Nacional, Costa Rica., Heredia, Costa Rica,* [7]*Programa de Investigación en Enfermedades Tropicales, Escuela de Medicina Veterinaria, Universidad Nacional, Costa Rica., Heredia, Costa Rica,* [8]*Laboratorio de Bioseguridad, Laboratorio Nacional de Servicio Veterinario, Servicio Nacional de Salud Animal, Ministerio de Agrivultura y Ganadería, Costa Rica., Heredia, Costa Rica,* [9]*Integrative Ecology Marine Department, Anton Dohrn Zoological Station, Italy., Heredia, Costa Rica*

Wildlife pathogen agents are of increasing concern worldwide because they threaten not only wildlife populations but also human and domestic animals health. Reporting of disease data for wildlife in biodiverse hot spot countries allow the early detection of infectious agents enforcing disease control, management and preventive medicine measurements. This assessment was dedicated to passive monitoring surveillance system of selected pathologic agents in free-ranging terrestrial mammals, reptiles and birds casualties recorded during a two-years period time. The study main to assess the more important zoonotic diseases reported in Costa Rica, including viral agents (rabies, influenza, flavivirus, alphavirus), bacterial agents (mycobacteriosis, salmonellosis), parasites (angiostrongyliasis, baylisascariasis, gnathostomiasis, leishmaniosis, trypanosomiasis, toxoplasmosis), among other pathogenic agents. Gross examination and histopathological findings were identified in 74 out 82 vertebrates showing some changes associated with the pathological alteration. Of total 68 assessed agents, 43 % of them were zoonotic. Macroparasites such as *Angiostrongylus costarricensis, Baylisascaris procyonis* and *Gnathostoma turgidum* were detected (13%) in peri-urban natural hosts. *Prosthenorchis elegans* was determined causing fatal gastrointestinal pathologies in 60% of *Saimiri oerstedii,* whereas filariasis shown a 33% infection in mammals under 500 meters above sea level life zone. Canine distemper virus was the most common diagnosed virus (7/74) affecting mainly procyonids, besides a suspicious case of WNV in pelicans is under investigation. Potentially zoonotic bacteria (6%) as *Staphylococcus aureus, Klebsiella pneumoniae* and *Clostridium perfringens* were diagnosed. This study demonstrates the need for the development of an integrated wildlife disease surveillance and monitoring system for the management of the disease in free-ranging wildlife in Costa Rica.

917

LIVESTOCK TICKS AND RISK FACTORS LINKED TO TICK INFESTATION IN CATTLE IN PERI-URBAN LIVESTOCK FARMING IN THE DISTRICT OF ABIDJAN AND THE MUNICIPALITY OF AZAGUIA (SOUTH AND SOUTH-EAST OF CÔTE D'IVOIRE)

Claude Aimée Amenan Diaha-Kouame[1], Valery Edgard Adjogoua[1], Negnerogo Guindo-Coulibaly[2], Marc Hermann Akaffou[1]

[1]*Institut Pasteur de Côte d'Ivoire, Abidjan, Côte D'Ivoire,* [2]*Felix Houphouet Boigny University, Abidjan, Côte D'Ivoire*

Ticks born diseases pose a problem of public health in the world and particularly in sub-Saharan Africa where the majority of the affections is due to malaria and many other diseases of viral, parasitic or bacterial origins. The aim of this study was to identify tick species of cattle in cattle farms and to determine the possible risk factors linked to tick infestation in the district of Abidjan and the commune of Azaguié. Thus, in July 2019, thirteen (13) herds distributed in these localities were visited for the removal of ticks and to conduct epidemiological surveys. On each pass, ticks were harvested from 15 cattle per herd. All the farms visited were infested with ticks. Of all animals sampled, 96.92% had ticks. The total number of ticks collected was 1796, of which 89.42% (1606) were adults, 10.41% (187) were nymphs and 0.17% (3) were larvae. Two tick species have been identified, these are *Amblyomma variegatum* with 25% of the numbers and *Rhipicephalus (Boophilus) microplus* with 75%. 96% of the oxen were infested with ticks of the species *Rhipicephalus (Boophilus) microplus* and 56% were infested with ticks of the species *Amblyomma variegatum*. The co-infestations of cattle by the two identified species were 53%. The distribution of the sexes has shown that in *Amblyomma variegatum* species the males are more numerous with (13.44% for the male and 8.76% for the females). In contrast, in *Rhipicephalus (Boophilus) microplus* species, females were more numerous (5.08% for males and 62.3% for females). Analysis of the risk factors associated with tick infestation of cattle has shown that factors such as poorly demarcated pens, animal feeding methods, lack of knowledge of the application of acaricides contribute to major ectoparasitic infestations in these animals. Samples of ticks collected from peri-urban farms in the district of Abidjan and the locality of Azaguié as part of this study indicate that the relatively recent introduction of the species *Rhipicephalus (Boophilus) microplus* poses a threat to the animal and human health.

918

THE ROLE OF DOMESTIC ANIMALS AND RODENTS IN THE ECO-EPIDEMIOLOGY OF RICKETTSIAS IN THE PERUVIAN AMAZON BASIN

Cusi Ferradas[1], David E. Payahuanca[1], Diana León[1], Edith Malaga[2], Gabriela Salmón-Mulanovich[3], Andrés G. Lescano[1], Janet E. Foley[4]

[1]*Emerging Diseases and Climate Change Research Unit, School of Public Health, Universidad Peruana Cayetano Heredia, Lima, Peru,* [2]*Infectious Diseases Laboratory, School of Science, Universidad Peruana Cayetano Heredia, Lima, Peru,* [3]*Biomedical Engineering Program PUCP-UPCH, Universidad Peruana Cayetano Heredia (UPCH), Pontificia Universidad Católica del Perú (PUCP), Lima, Peru,* [4]*Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, Lima, Peru*

Rickettsias are intracellular bacteria transmitted from animals to humans by ectoparasites (ticks, fleas, lice, and mites). In Iquitos, the main city of the Peruvian Amazon basin, the seroprevalence of Spotted Fever Group and Typhus Group rickettsias in humans ranges between 20.0-43.0% and 2.8-6.2%, respectively. However, there is less data on rickettsial ecology in rural areas, where seroprevalence has not been determined and the identities of animals acting as reservoirs of these bacteria are not known. We conducted a cross-sectional study in Zungarococha, a rural community located 45 minutes away from Iquitos, to better understand the epidemiology and ecology of rickettsial diseases in the Peruvian Amazon Basin. We collected blood samples from humans, blood samples and ectoparasites from dogs and cats, and blood, ectoparasites, and tissue samples form peridomestic rodents. We screened ectoparasites and rodent tissues by real time-PCR (qPCR) targeting the rickettsial conserved gene *gltA*. Blood samples from dogs, cats, and humans were analyzed by Indirect Immunofluorescence Assays (IFA) to detect antibodies against rickettsias. 56 (98.3%) and 4 (100%) of pools of *Ctenocephalides felis* fleas collected from dogs and cats were PCR-positive, respectively. Of the 52 *Rhipicephalus sanguineus* ticks collected from 14 dogs, 2 (14.3%) of the pools were PCR-positive. All 15 rodent tissue samples were PCR-negative. IgG seroprevalence was 58.8% (40/68) for dogs, 0% (0/4) for cats, and 52.4% (11/21) for humans. Our results indicate that rickettsias are circulating among dogs, cats, their ectoparasites and humans in rural areas of the Amazon Basin. The study is ongoing, and we will continue capturing rodents in order to determine if they have a role in the transmission cycle of rickettsia as has been reported in other geographic areas.

919

URBAN PROCYONID, DOMESTIC DOGS AND HUMAN INTERACTION AS RISK FOR CONTAGION OF THE ZOONOTIC NEMATODES *ANGIOSTRONGYLUS COSTARICENSIS* AND *BAYLISASCARIS PROCYONIS* IN COSTA RICA

Alejandro Alfaro-Alarcón[1], Mario Santoro[2], Mario Baldi[3]

[1]*Escuela de Medicina Veterinaria Universidad Nacional, Heredia, Costa Rica,* [2]*Integrative Marine Ecology Department Stazione Zoologica Anton Dohrn, Naples, Italy,* [3]*Programa de Investigación en Enfermedades Tropicales Escuela de Medicina Veterinaria Universidad Nacional, Heredia, Costa Rica*

Close proximity and interaction between procyonids, humans and domestic animals is a growing phenomenon that happens in human settlements, which frequently ends up in human-wildlife disease transmission. *Angyostrongylus costaricensis* and *Baylisascaris procyonis* are important zoonotic agents in periurban wild animals in Costa Rica. In a seven-year period, 252 specimens of the Procyonidae family, associated with urban areas, were analysed. 102 *Nasura narica* and 150 *Procyon lotor* underwent post-mortem examination. First-stage larvae of *A. costaricensis* from feces were recovered by Baermann technique. Angyostrongylus identity was confirmed by morphological features of adult parasites and molecular characterization of specific amplification of cytochrome c oxidase subunit 1 (cox1) and 18SrRNA gene fragments of adult worms. The overall prevalence was 85.3 % of animals (*Nasua narica*) were positives to *A. costaricensis*, showing a severe pyogranulomatous enteritis and granuloma formation in the mesenteric arteries. The local prevalence in different geographic areas showed a variability between 2.3% and 87.3%. 35.5% of *P. lotor* with presence of Baylisascaris were associated with catarrhal enteritis. Parasite identification was performed by adult nematode morphological features and molecular identification of DNA sequency from mitochondrial cytochrome c oxidase 2 gene and the ribosomal ITS1–5.8S-ITS2, and ribosomal 28S genes. Co-infestation of *A. costaricensis* and *Baylisascaris procyonis* was observed in ten out of 150 Raccoons. Baylisascaris prevalences were between 0 to 60.3%. Six cases of housed dogs were also analysed and showed gastrointestinal and hepatic disease associated to *A. costaricensis*. The increased interactions between humans, wildlife and domestic animals in urban areas highlight the need to understand the role of these two species of peri-urban procyonids as potential spreaders and reservoirs of those parasites. These findings also call for a one health approach in order to reduce the risk of disease spillover in highly populated areas in Costa Rica.

920

IDENTIFYING AREAS AT HIGH RISK FOR DOG MEDIATED RABIES IN THAILAND

Kaushi ST Kanankege[1], Kaylee M. Errecaborde[1], Anuwat Wiratsudakul[2], Ong-orn Prasarnphanich[3], Phrutsamon Wongnak[4], Chakchalat Yoopatthanawong[2], Julio Alvarez[5], Andres M. Perez[1]

[1]*University of Minnesota, St Paul, MN, United States,* [2]*Faculty of Veterinary Science, Mahidol University, Nakhon Pathom, Thailand,* [3]*United States Centers for Disease Control and Prevention (CDC) Thailand Office, Nonthaburi, Thailand,* [4]*Université Clermont Auvergne, INRAE, VetAgro Sup, UMR EPIA, Saint-Genès-Champanelle, France,* [5]*Centro de Vigilancia Sanitaria Veterinaria (VISAVET), Universidad Complutense, Madrid, Spain*

Despite immense efforts, rabies remains an endemic and neglected zoonotic disease in Thailand. To evaluate and improve the effectiveness of control programs, it is imperative for policymakers and program managers alike to understand the spatiotemporal patterns of rabies spread in relation to underlying risk factors. Here, we generated rabies risk maps for Thailand using a geographical analysis approach that incorporated data on human and animal rabies cases and underlying epidemiologically important factors hypothesized to influence the risk of the disease. A zero-inflated Poisson regression and conditional autoregressive (CAR) models were fitted to account for the spatial dependence between districts while accounting for the statistically significant variables. Our results suggest that the number of dog bites/attacks, the total number of owned and un-owned dogs, sharing country borders, number of Buddhist temples, and poverty levels were the key factors associated with human or dog rabies in Thailand. The CAR model cross-validation indicated fair performance with 0.77 AUC and the fitted values of the CAR model was used to develop risk maps. While subject to limitations, we believe that this work could be useful as a guide when planning risk-based management approaches to improve current rabies control efforts in Thailand including the distribution of human Post Exposure Prophylaxis and antirabies vaccines for animals.

921

PREVALENCE OF PROXIMATE RISK FACTORS OF ACTIVE TUBERCULOSIS IN LATENT TUBERCULOSIS INFECTION: A CROSS-SECTIONAL STUDY FROM SOUTH INDIA

Saravanan Munisankar, Anuradha Rajamanickam, Suganthi B, Sathiswaran Muthusamy, Chandra Kumar Dolla, Pradeep Menon, Thomas Nutman, Subash Babu

NIRT-NIH-ICER, Chennai, India

The prevalence of proximate risk factors for active tuberculosis (TB) in areas of high prevalence of latent TB infection (LTBI) are not clearly understood. We aimed to assess the prevalence of non-communicable multimorbidity focusing on diabetes, malnutrition, and communicable co-morbidity of helminth infections- *Strongyloides stercoralis* (Ssl) and filarial infection as common risk factors of LTBI progressing to active (TB). In a cross-sectional study, 2351 adults (45% male and 55% female) from villages in Kancheepuram district of South India were enrolled from November 2012 to March 2019. Diabetes was defined as HbA1c of ≥6.4%, undernutrition was defined as body mass index (BMI) <18.5 kg/2, and LTBI was defined as positive (≥ 0.35 international units/ml) by QuantiFERON Gold In-Tube assay. A total of 1226 samples (52%) were positive for LTBI out of 2351 tested samples. The prevalence of diabetes (DM) and pre-diabetes (PDM) was 20% (246/1226, 95%CI: 0.9-1.4%), and 35% (429/1226, 95%CI: 1.0-1.4%), respectively. Moderate/severe undernutrition was 9% (108/1226, 95%CI: 0.9-1.7%). Asymptomatic Ssl was 35% (423/1226, 95%CI: 1.0-1.4%) and asymptomatic filarial infection was 1%. The association of undernutrition (Adjusted Odds Ratio (AOR) = 1.32, 95%CI: 0.9-1.8), DM and PDM (AOR = 1.0, 95%CI: 0.85-1.37; AOR = 1.18, 95%CI: 0.97-1.42), and Ssl (AOR = 1.12, 95%CI: 0.94-1.34) strongly pose as risk factors of LTBI progression to active TB. Males exhibit (AOR = 1.48, 95%CI: 1.25-1.75) a high chance of possessing risk of multimorbid factors. The present evidence of a high burden of multimorbidity suggests that proximate risk factors of active TB in LTBI can be managed by nutrition and lifestyle modification.

922

IMPACT OF THE INTRODUCTION OF PCV7/13 ON ANTIMICROBIAL RESISTANCE IN INVASIVE PNEUMOCOCCAL DISEASE IN SENEGAL

Ebrima Bah, Sang Jatta

Ministry of Health and Social Welfare, Banjul, Gambia

We describe antimicrobial resistance in invasive pneumococcal due to all serotypes and non-vaccine type (NVT) pre and post pneumococcal conjugate vaccine (PVT) implementation in Senegal in all age groups. We identify, serotype, and performed antimicrobial susceptibility testing using disc diffusion methods on pneumococcal isolates obtained from invasive samples collected from standardized population-based pneumococcal disease surveillance in the Casamance & demographic surveillance system. The study commenced May 2012. Pcv7 was introduced in august 2013 and pvc13 in May 2016. Antibiotic susceptible were interpreted using clinical laboratory standard institute guidelines. 500 pneumococcal isolates were screened against five antimicrobial agents. There was a moderate decline in antibiotic resistance in all age groups in invasive pneumococcal disease during vaccine implementation. In the 2 months to 2-year age group, annual counts of oxacillin. Chloramphenicol, and tetracycline resistant cases fell from 10-15 in 2013 & 2014 to 6-7in 2017 & 2018. In the 24-59-month age group, there was a large fell in tetracycline resistance cases.in those >5 years, oxacillin, chloramphenicol, and tetracycline resistance fell to zero cases in 2015 & 2016. Resistance due to reductions in vaccine-serotypes 1, 5,14 & 23f. The proportion of resistant NVT cases increase over time, particularly in the 2-23-month age group, with tetracycline resistance mainly in serotypes 10A, 12F, 11B, 7C and 25A & tetracycline resistance in serotype12F in 2016. Isolates were generally sensitive to erythromycin but 95-98 were generally sensitive to cotrimoxazole throughout the study. Although there is an overall reduction in case of antimicrobial resistance IPD, resistance is emerging in NVT. We hypothesis that increased transmission of NVT after the introduction of PCV and exposure to antimicrobials facilitates the emergence of resistance in NVT. Ongoing surveillance is important to determine future trends in resistance as it has both clinical and public health importance in PCV era.

923

LINE PROBE ASSAYS DETECTION OF FIRST- AND SECOND-LINE DRUG RESISTANCE IN EXTRAPULMONARY TUBERCULOSIS: A RETROSPECTIVE ANALYSIS

Aislinn K. McMillan[1], Swapna Naik[2], Rebecca E. Colman[1], Marva Seifert[1], Mubin Kazi[2], Anjali Shetty[2], Timothy C. Rodwell[1], Camilla Rodrigues[2]

[1]*Department of Medicine, University of California, San Diego, La Jolla, CA, United States,* [2]*Section Microbiology, Department of Laboratory Medicine, P.D. Hinduja National Hospital and Medical Research Center, Mumbai, India*

It is estimated that 15% of all tuberculosis (TB) is extrapulmonary. Clinical presentation of extrapulmonary TB (EPTB) is diverse, often leading to misdiagnosis and delayed treatment. Early detection of *Mycobacterium tuberculosis (Mtb)* and drug resistance is a priority to enable early and appropriate TB treatment. Hain line probe assays (LPAs) are rapid molecular diagnostics that can detect *Mtb* and both first- and second-line drug resistance in <1 day, but they have not been WHO- endorsed for EPTB diagnosis. We describe the performance of Hain LPAs on EPTB clinical specimens at a tertiary care center in Mumbai, India. Hospital diagnostic registers were reviewed to identify samples of patients at risk for EPTB that underwent routine LPA analysis from January 2015 through June 2019. LPA, MGIT 960 culture, phenotypic drug susceptibility testing (DST), and acid-fast bacilli (AFB) smear results for extrapulmonary clinical samples were extracted and collated. Performance of LPA on clinical samples was compared to DST by computing sensitivity, specificity, positive predictive

value (PPV), and negative predictive value (NPV) for rifampicin, isoniazid, ofloxacin, moxifloxacin, kanamycin, amikacin, and capreomycin. Of the 256 samples included in this study, 55.9% were smear negative, 33.6% were scanty, and 10.5% were smear positive. A total of 399 LPA assays, a combination of first- and second-line drug screenings, were run on the 256 samples, of which 310 assays (77.7%) produced valid LPA results. The sensitivity, specificity, PPV, and NPV of the LPA tests for drug-resistance detection compared to phenotypic DST ranged from 94.4-100.0%, 80.0-100.0%, 0.60-1.00, and 0.94-1.00, respectively. LPA performance on EPTB samples was similar to LPA performance on sputum samples regardless of smear status. Of the samples that gave invalid or indeterminate readings on LPA, most were negative or scanty AFB smear status, suggesting the results could likely be attributed to pauci-bacillary load. These data suggest that LPA has true diagnostic potential to provide rapid and accurate results on clinical EPTB samples despite the low bacterial load.

924

USE OF AN ACUTE FEBRILE ILLNESS ENHANCED SURVEILLANCE SYSTEM TO MONITOR CONCURRENT RESPIRATORY AND ARBOVIRAL DISEASE TRENDS IN PUERTO RICO DURING THE NOVEL CORONAVIRUS PANDEMIC, 2020

Hannah R. Volkman

Centers for Disease Control and Prevention, San Juan, Puerto Rico

The Sentinel Enhanced Dengue Surveillance System (SEDSS) is an active surveillance system that recruits participants with acute febrile illnesses in two tertiary care hospitals and one outpatient clinic in Ponce and San Juan, Puerto Rico. SEDSS monitors infectious disease trends and serves as an outbreak detection early warning system. SEDSS participants are tested for several arboviral and respiratory pathogens; testing for the 2019 novel coronavirus, SARS-CoV-2, began for respiratory samples collected February 16 and later. Upon recognition of community transmission of SARS-CoV-2 in Puerto Rico, Emergency Department (ED) visit data were also extracted weekly from SEDSS hospital medical records systems to monitor syndromic trends in febrile and respiratory illness complaints, as well as ICD-10 diagnoses related to COVID-19. A dynamic data dashboard was constructed to visualize laboratory and syndromic trends in real time with interactive stratification by location, age group, and symptoms. Concurrent with a territory-wide curfew beginning March 15, SEDSS enrollments decreased from an average of 90.5 enrollments (St Dev. 58.8-122.1) per week in 2020 prior to the curfew, to 23.0 enrollments (St Dev. 9.5-36.5) per week during March 15-April 11. Prior to the curfew, 27.6% of 978 samples were positive for influenza A or B, 4.2% were positive for other (non-SARS-CoV-2) respiratory pathogens, 2.2% were positive for dengue, and no SARS-CoV-2 was detected. From March 15-April 11, 8.3% of 84 samples were positive for influenza A or B, 3.6% were positive for SARS-Cov-2, 3.6% were positive for other respiratory pathogens, and 1.2% were positive for dengue. Longer-term findings and syndromic trends are forthcoming due to the ongoing and evolving nature of the novel coronavirus pandemic. SEDSS is uniquely positioned to identify trends, describe clinical outcomes, and differentiate arbovirus and respiratory virus etiologies causing acute febrile illness. This platform is being leveraged during the novel coronavirus pandemic to inform evidence-based public health action, clinical decision making, and prevention messaging in Puerto Rico.

925

THE PREVALENCE OF *MYCOBACTERIUM TUBERCULOSIS* AMONG ACID FAST CULTURES FROM MILITARY HEALTH SYSTEM BENEFICIARIES FROM HAWAII AND PACIFIC ISLANDS FROM JANUARY 2002 TO NOVEMBER 2019

Elena M. Crecelius, Michael B. Lustik, Timothy S. Horseman, Milissa U. Jones

Tripler Army Medical Center, Honolulu, HI, United States

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB), is the leading infectious cause of death worldwide and Hawaii (HI) has the second highest case rate of TB in the United States. The prevalence of TB among military health system (MHS) beneficiaries (active duty service members, retirees, dependents, civilians and eligible Pacific Island civilians) in HI has not been previously reported. Our analysis evaluates the prevalence of MTB among acid fast culture(s) (AFC) tested at Tripler Army Medical Center (TAMC) on Oahu, HI and describes demographic factors associated with positive samples. We analyzed AFC results from TAMC's clinical diagnostic microbiology laboratory from January 2002 to November 2019. Demographic data were recorded for each individual with an AFC result during the study period. Prevalence was calculated based on the number of MTB-positive AFC per all AFC over the study period. Multivariable logistic regression was used to evaluate associations between demographic factors and MTB-positive AFC results. There were 4768 AFC, belonging to 4482 persons, resulted at TAMC from January 2002 to November 2019. There were a total of 49 MTB-positive AFC, belonging to 44 persons, resulting in a cumulative prevalence of 1.03 percent (Fig.). After controlling for other factors, Asian-Pacific Islanders had nearly 15 times higher odds of having a positive AFC than whites (OR=14.96, 95% CI 5.03, 44.55, p=<0.001) and active duty personnel had 2.6 times the odds of having a positive AFC than dependents, civilians and retirees (OR=2.6, 95% CI 0.94, 7.22, p=0.067). The low prevalence of MTB among AFC performed at our institution over nearly 16 years suggests that living in the state of HI does not appear to confer high rates of TB to MHS beneficiaries. Persons with Asian-Pacific Islander ethnicity have higher odds of positive AFC which corroborates prior studies regarding risk factors for MTB. Further analysis is needed to better understand why active duty personnel demonstrate higher odds of positive AFC than other MHS beneficiaries in HI. Follow-up analysis is underway to describe the clinical course of the 44 persons with MTB-positive AFC.

926

POTENTIAL USE OF RAPID, POINT-OF-CARE DIAGNOSTICS YO REDUCE ANTIBIOTIC PRESCRIPTION RATES AMONG PEDIATRIC PATIENTS PRESENTING WITH RESPIRATORY ILLNESS IN SOUTHWESTERN UGANDA

Emily J. Ciccone[1], Lydia Kabugho[2], Emmanuel Baguma[2], Rabbison Muhindo[2], Jonathan J. Juliano[1], Edgar Mulogo[2], Ross M. Boyce[1]

[1]*University of North Carolina, Chapel Hill, NC, United States,* [2]*Mbarara University of Science and Technology, Mbarara, Uganda*

Pediatric febrile acute respiratory illness (ARI) is a common reason for outpatient department (OPD) evaluation in sub-Saharan Africa. With widespread uptake of rapid tests, it is known that malaria accounts for only a small subset of these illnesses. Similar diagnostics for ARI are not routinely available, leading to frequent treatment with antibiotics. Point-of-care (POC) biomarkers, such as C-reactive protein (CRP) and procalcitonin (PCT), and pathogen-specific testing may help identify patients in whom antibiotics are not needed. To characterize pediatric ARI in southwestern Uganda, we enrolled 225 children aged 1-10 years presenting to Kasese Health Centre (KHC) OPD between October 2019 and January 2020 with fever for ≤7 days, ARI symptoms (cough, oxygen saturation (O2sat) <90%, and/or subjective fast breathing or respiratory rate (RR) ≥30 breaths per minute (bpm)), and a negative malaria test. Clinicians performed exams and prescribed treatment per local guidelines. Lab staff conducted the study tests. The median age was 3 years (IQR: 2-5) and 57% were female. The median fever duration was 5 days (IQR: 5-5); 15% had an axillary temperature >38.0°C on presentation. Cough (98%) and rhinorrhea (96%) were common; subjective fast breathing (8%) and chest in-indrawing (0.4%) were not. Few (3%) were hypoxic (O2sat < 90%), but many (68%) were tachypneic (RR >40bpm (ages 1-5), >30bpm (ages 5-10)). Only 13% (26/222) had a CRP ≥40mg/L and 3% (4/222) ≥80mg/L; 11% (14/126) had a PCT >0.5ng/mL. By rapid antigen test, 18% (40/225) were positive for influenza; 19 had type A and 21 type B. Only 7% of children were admitted. All but one were prescribed antibiotics. If reserving antibiotics for those with hypoxia and CRP >40mg/L, treatment could have been avoided in 85% (34/40) of influenza-positive and 84% (155/185) of influenza-negative children. In sum, most children presenting to KHC with malaria-negative, febrile ARI had mild illness (no danger signs, hypoxia,

or need for admission, and low CRP and PCT), yet nearly all received antibiotics. POC biomarker and pathogen testing may be useful to improve antibiotic stewardship in resource-limited settings.

927

STREPTOCOCCUS PNEUMONIAE IS AN IMPORTANT CAUSE OF BACTERIAL LOWER RESPIRATORY TRACT INFECTION IN SOUTHERN PROVINCE, SRI LANKA

Coralei E. Neighbors[1], Sky Vanderburg[1], Gaya B. Wijayaratne[2], Champica K. Bodinayake[2], Ajith Nagahawatte[2], Vasantha Devasiri[2], Ruvini Kurukulasooriya[2], Bhagya Piyasiri[3], Muhunthan Sellathurai[3], Thilini Wickremasinghe[2], Thishara Nanayakkara[2], Tianchen Sheng[1], Jack Anderson[1], Bradly P. Nicholson[4], L. Gayani Tillekeratne[1], Christopher W. Woods[1]

[1]*Duke University, Durham, NC, United States,* [2]*Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka,* [3]*Teaching Hospital Karapitiya, Galle, Sri Lanka,* [4]*Institute for Medical Research, Durham, NC, United States*

Lower respiratory tract infection (LRTI) is the most common infectious cause of death worldwide. The most common bacterial agent for LRTI is *Streptococcus pneumoniae*. The conjugate pneumococcal vaccine protects against *S. pneumoniae* but is not available through the public healthcare sector of Sri Lanka. Data regarding LRTI burden due to *S. pneumoniae* is limited in Sri Lanka. We conducted a prospective cohort study of patients presenting with LRTI to the largest tertiary care hospital in Southern Province, Sri Lanka, from January 2018 through December 2019. Consecutive patients ≥1 year old who met a case definition for LRTI with signs and symptoms of acute respiratory illness (e.g., cough) and acute infection (e.g., fever) were enrolled. Urine samples and sociodemographic and clinical information were obtained from patients. Urine was tested for *S. pneumoniae* antigen using Alere BinaxNOW™ *S. pneumoniae* Antigen Cards. Bivariable analyses with the Fisher exact and Kruskal-Wallis tests were performed to identify demographic and clinical features associated with *S. pneumoniae* urine antigen detection. In total, 393 patients were enrolled during the study period, 50.9% male. Overall, the median age was 56 years (IQR 25-69 years). Of the cohort, 46 (11.7%) tested positive for *S. pneumoniae* urine antigen. Of 342 (87.0%) patients who received chest x-rays, 164 (48.0%) had abnormal reading results with no association to antigen detection. Age under 5 years (21.7% vs. 7.8%, p= 0.006) and immunosuppression within the last 30 days (13.0% vs 0.0%, p< 0.001) were associated with antigen detection. Fever was associated with *S. pneumoniae* (93.5% vs 72.3%, p=0.006). Patients with LRTI listed as a discharge diagnosis were more likely to have antigen detection (70.0% vs 44.1%, p=0.001). Antigen detection was associated with the prescription of antibiotics (73.5% vs 51.6%, p=0.017). The overall median hospital duration was 3 days and 2 participants who tested negative for *S. pneumoniae* died. Our findings suggest that *S. pneumoniae* is an important cause of bacterial LRTI in Sri Lanka and more research on the implementation of the pneumococcal vaccine is warranted.

928

CAN XPERT ULTRA USING STOOL SPECIMEN HELP IN UNDER-FIVE CHILDHOOD TB DIAGNOSIS?

Senjuti Kabir[1], S. M. Mazidur Rahman[1], Sabrina Choudhury[1], Shakil Ahmed[2], Rumana Nasrin[1], Shahriar Ahmed[1], Mohammad Khaja Mafij Uddin[1], Razia Khatun[1], Mohammod Jobayer Chisti[1], Sayera Banu[1]

[1]*icddr,b, Dhaka, Bangladesh,* [2]*Shaheed Suhrawardy Medical College and hospital, Sher-E-Bangla Nagar, Dhaka, Bangladesh*

Children, especially under-five years old, are more at risk to develop severe forms of tuberculosis (TB) and hence, progression to death is higher. Inability to expectorate sputum, difficulty in respiratory specimen collection, paucibacillary nature of TB disease and lack of gold standard diagnostic are common barriers to detect childhood TB (ChTB). A convenient specimen along with highly sensitive diagnostic is required for better ChTB diagnosis. We evaluated the performance of Xpert MTB/RIF Ultra assay (Ultra) using stool for TB detection in under-five children. We enrolled presumptive under-five ChTB cases admitted in four hospitals, Dhaka between January'18 and April'19. Induced sputum (IS) and stool were collected from each enrolled children to perform smear microscopy, solid culture and Ultra. Children, positive in any test on either specimen, were considered as "bacteriologically positive (B+ve) case", that on IS as "B+ve case on IS" and on stool as "B+ve case on stool". Children with clinical suspicions and negative test results were diagnosed clinically by treating physicians. Proportion of positivity of Ultra using IS and stool was compared. At 95% confidence interval, sensitivity, specificity and accuracy of Ultra using stool were measured considering "B+ve case on IS" as reference standard. Median age of the enrolled 296 presumptive ChTB cases was 11.8 months (IQR: 7.1-25.5). Of 66 (22.3%) TB cases identified, 44 (66.7%) were B+ve. By Ultra, 14 (31.8%) of B+ve cases were identified by IS while 36 (81.8%) identified by stool (p<0.001). Ultra detected all B+ve stool specimens (n=36) and 32 (88.9%) were "trace call". One IS was positive in culture but negative by other tests. Among "B+ve cases on IS" (n=15), Ultra using stool had sensitivity (46.7%), specificity (89.7%) and accuracy (87.5%). Ultra exclusively identified 35 (97.2%) of "B+ve cases on stool". Moreover, Ultra using stool detected 7.4% additional B+ve cases. Stool, an easy-to-get specimen, was found significantly superior than IS for under-five ChTB detection using Ultra. Future studies are required to validate these findings and manage the children with "trace call".

929

SPUTUM MICROBIAL PROFILE AND CLINICAL FEATURES OF PATIENTS WITH GENEXPERT AND AFB NEGATIVE IN SAN LAZARO HOSPITAL MANILA-PHILIPPINES

Crespoo Mbe-cho Ndiabamoh

TMGH, Nagasaki, Japan

The Philippines is amongst the seven countries accounting for 90% of the burden of tuberculosis (TB) in the world in 2019. We sought to investigate the presence of other potentially pathogenic bacteria and the clinical features in archived sputum samples of acutely unwell patients in the TB ward in Manila and presenting with symptoms consistent with a clinical diagnosis of pulmonary TB, but negative for Xpert(®) MTB/Rif assay and Acid Fast Bacilli. A retrospective analytical cross-sectional study on 82 sputa samples was done. DNA extracted using a QIAGEN kit. Positive controls prepared from blood culture isolates were extracted using the same kit following protocol. Three multiplex Polymerase chain reactions (PCR) for 16S rRNA assays were designed for typical bacteria (*Streptococcus pneumoniae, Hemophilus influenzae, Moraxella catarrhalis*), atypical bacteria (*Chlamydia pneumoniae, Legionella pneumoniae, mycoplasma pneumoniae*) and (*Burkholderia pseudomallei, Burkholderiathailandensis*). Final PCR products were run in Shimadzu MultiNA Microchip Electrophoresis System for DNA analysis. All data were analyzed using STATA®15. Overall bacteria prevalence was 74/82(90.24%). The most prevalent were *Burkholdera pseudomallei* 72/82(87.80%) and *Hemophilusinfluenza*22/82(26.83%). A high proportion of patients had a cough of greater than 2 weeks 51/82(76.12%) and chest x-ray findings consistent with TB infection 59/76(86.76%). The odds of having a cough for more than two weeks and weight loss were about five {OR 5.70(CI: 1.79, 18.09)}, p=0.0008 and four times{OR 4.44(95%CI:1.56, 12.62)} p=0.0022 respectively higher in those with positive Xpert/AFB compared to those negative. On the other hand, there was no significant association observed (p= 0.1006) with chest x-ray lesions consistent with TB. The prevalence of other bacterial infections is high in patients suspected and/or diagnosed with PTB although this warrants more research on the types and prevalence of each. Most clinical features such as cough over 2 weeks, chest x-ray lesions, fever, and weight loss can be present in other lung affections/diseases.

930

MOLECULAR CHARACTERISTICS OF INFLUENZA A STRAINS IN KENYA AND CROSS REFERENCING TO RECOMMENDED VACCINE STRAINS

Faith Sigei[1], Gathii Kimita[1], Christian K. Fung[2], Irina Maljkovic M. Berry[2], John N. Waitumbi[1]

[1]*Basic Science Laboratory, US Army Medical Research Directorate - Africa, Kisumu, Kenya,* [2]*Viral Diseases Branch, Walter Reed Army Institute of Research, Silver Spring, MD, United States*

Kenyan Influenza viruses circulate year-round with peaks from June to October, with Influenza A contributing a notable fraction of these infections. Two Influenza A strains, namely H1N1 and H3N2 are the currently circulating seasonal influenza A virus subtypes. They evolve rapidly due to antigenic drift, a phenomenon that contributes to generation of novel viral strains. It is therefore essential to monitor the circulating strains of flu to inform vaccination policy. Nasopharyngeal swabs collected from febrile patients between 2017 and 2019 at diverse surveillance sites across Kenya were screened for respiratory viruses including Influenza A virus using a multiplex real time PCR. 108 influenza A samples were subtyped and then sequenced using a targeted amplicon sequencing approach. All Kenyan H1N1 strains clustered with A/Brisbane/02/2018, the recommended 2020 Southern Hemisphere vaccine strain. Kenyan H3N2 strains presented as two clades. Clade one strains matched the A/Southern Australia/34/2019 vaccine strain recommended for the 2020 Southern Hemisphere flu season. Clade 2 strains were not represented in the recommended 2020 Southern Hemisphere vaccine. This clade clustered with the A/Switzerland/9715293/2013 strain that was a component of the 2015 Southern Hemisphere flu season vaccine. Conclusion, Our results indicate that, while the circulating H1N1 and some H3N2 strains were well matched to the recommended current vaccine strains, some strains from the 2015 influenza season are currently in circulation in Kenya, posing a false sense of protection in light of the 2020 vaccine formulation which no longer includes 2015 strains. Our findings indicate the need to monitor the circulating strains to inform on the vaccine formulations and to ensure effectiveness of seasonal influenza vaccines.

931

RESPIRATORY TRACT MICROBES AND ASSOCIATED FACTORS IN PULMONARY TUBERCULOSIS PATIENTS AT BOBO-DIOULASSO

Nina Gouba[1], Moussa Sakana[2], Assana Cissé[3], Kader Ilboudo[3], Nadège W. Somda[3], Zékiba Tarnagda[3]

[1]*Université Nazi Boni de Bobo-Dioulasso, Institut de recherche en science de la santé DRO- Bobo-Dioulasso, Bobo-Dioulasso, Burkina Faso,* [2]*Centre médical avec Antenne Chirurgical du district sanitaire de Do, Bobo-Dioulasso, Bobo-Dioulasso, Burkina Faso,* [3]*Institut de recherche en science de la santé DRO- Bobo-Dioulasso, Bobo-Dioulasso, Burkina Faso*

The human respiratory tract is an ecosystem of commensals and potential pathogen microbes. Few studies investigated on respiratory microbes among TB (Tuberculosis Bacillus) patients. Herein we used multiplex PCR to investigate the microbes and others associated factors in pulmonary tuberculosis patients at Bobo-Dioulasso. A total of 85 TB suspected patients of pulmonary tuberculosis and 51 controls without TB both presenting a severe acute respiratory infection (SARI) has been enrolled in two medical centers at Bobo-Dioulasso. Sputum samples were collected from TB suspected and diagnostic based on microscopy of Ziehl-Neelsen sputum staining. Nasopharyngeal and oropharyngeal samples collected in swabs from confirmed TB patients and controls enrolled. DNA was extracted using the QIAamp® Viral RNA kit and amplification was performed with Real time RT Multiplex PCR using FTD Resp 33 kit. An analyse by logistic regression was used to access factors associated with TB. The overall median age of all study participants was 40 years, 44.12% age range (35-59 years) and 66.91% (91/136) were men. Some factors are statistically significantly found associated with TB status such as male sex (aOR 5.6, 95%CI: 1.71- 18.47, p=0.004), young adult (14-35 year) (aOR 5.1, 95%CI: 1- 22.9 p=0.040) and *Staphylococcus aureus* (aOR 9.08, 95%CI: 1.15- 71.35, p=0.036). However, urban residence (aOR 0.35, 95%CI: 0.12-0.97, p=0.045) and *Streptococcus pneumoniae* carriage (aOR 0.09, 95%CI: 0.03- 0.25, p=0.000) infections were less likely to be TB infected. A total of 24 virus, 113 bacteria and 3 fungi were found in all participants. Virus were more prevalent in TB patients 18/24 (75%) compared to controls 33% (8/24). In contrast, bacteria were more prevalent in controls 73/113 (64.6%) than TB patients 40/113(35.4%) (p=0.008). However, there were no microbes found specific associated to TB status. The respiratory tract colonization by *S. pneumoniae* and young adults were less likely to have TB infection.

932

STREPTOCOCCUS PNEUMONIAE COLONIZATION AMONG CHILDREN IN GALLE, SRI LANKA: A CROSS-SECTIONAL STUDY

Chi Zhang[1], Gaya Wijayaratne[2], Sasiru Anukalana sapukotana[3], Champika Bodinayake[2], Ajith Nagahawatte[2], Joy Noel Baumgartner[1], Tianchen Sheng[1], Madureka Premamali[2], Ruvini Kurukulasooriya[2], Lawrence P. Park[1], Bradly P. Nicholson[4], Christopher Woods[1], Gayani Tillekeratne[1]

[1]*Duke University, Durham, NC, United States,* [2]*University of Ruhuna, Galle, Sri Lanka,* [3]*Teaching Hospital, Karapitiya, Galle, Sri Lanka,* [4]*Institute for Medical Research, Durham, NC, United States*

Streptococcus pneumoniae, a Gram-positive bacterium, is the most common cause of bacterial pneumonia globally. Pneumococcal pneumonia can be effectively prevented by pneumococcal vaccines, which are currently not provided through the public healthcare sector in Sri Lanka. We conducted a surveillance study to determine prevalence of *S. pneumoniae* colonization among healthy children in Sri Lanka. The cross-sectional study was conducted in Galle, Sri Lanka from July to September 2019. Eleven Medical Officer of Health clinics, centers providing routine vaccinations to infants and children, were visited. A nasopharyngeal sample was collected from each enrolled child and socio-demographic and clinical data were obtained by interviewing the parents. Routine microbiological testing was conducted to confirm the presence of *S. pneumoniae* isolates. Antibiotic susceptibility testing was performed on confirmed isolates using Kirby-Bauer disc diffusion. Socio-demographic and clinical characteristics associated with *S. pneumoniae* colonization were assessed using bivariable and multivariable logistic regression models. Among 123 enrolled children, the median age was 12 months (IQR 6-34) and 71 (57.7%) were male. Overall, 26 (21.1%) were colonized with *S. pneumoniae*. Higher risk of *S. pneumoniae* colonization was associated with living with other children <5 years in both bivariable and multivariable analyses (unadjusted OR=4.58, 95%CI: 1.69-12.83; adjusted OR=3.99, 95%CI: 1.19-13.39). Lower risk of colonization was found among children drinking boiled water on multivariable analysis (adjusted OR=0.11, 95%CI: 0.02-0.65). Non-susceptible prevalence was 94.4% to oxacillin/penicillin, 72.2% to erythromycin, and 44.4% to clindamycin. All isolates were susceptible to levofloxacin. This is the first report of *S. pneumoniae* colonization prevalence among children in Southern Province, Sri Lanka. Multi-drug non-susceptible pneumococcal colonization was common in children. Further population-level data regarding colonization and disease burden are needed to guide pneumococcal vaccine decisions in Sri Lanka.

933

EPITOPE SIGNATURES IN COVID-19 PATIENTS WITH MILD AND SEVERE DISEASE OUTCOMES

Kirsten Heiss[1], Tatjana Schwarz[2], Yuvaraj Mahendran[1], Renate Sekul[1], Christian Drosten[2], Volker Stadler[1], Victor Corman[2]

[1]*PEPperPRINT GmbH, Heidelberg, Germany,* [2]*Charité-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Institute of Virology, Berlin, Germany*

The worldwide ongoing transmission of COVID-19 is a major global health concern. The causative agent of this acute respiratory disease is a newly emerged coronavirus named SARS-CoV-2. The virus originated from China in late 2019 and rapidly spread across the globe. The course of the disease ranges from non-symptomatic to mild symptoms such as fever and cough to severe cases with pneumonia, acute respiratory distress and potentially death. Humoral responses are an important defense mechanism in viral infections. The investigation of antigens and/or epitopes recognized by SARS-CoV-2-specific antibodies is not only crucial for the development of intervention strategies, but also for epidemiological studies, disease prognosis and the identification of novel diagnostic markers. With the aim to decipher SARS-CoV-2-specifc humoral immune responses on the epitope level, we screened sera from COVID-19 patients with mild and severe symptoms using high-density peptide microarrays covering the entire proteome of SARS-CoV-2 as 15 amino acid peptides with an overlap of 13 amino acids. The high peptide-to-peptide overlap of our SARS-CoV-2 proteome array allowed a high-resolution epitope analysis giving a detailed picture of antibody binding patterns. In the present study, we describe distinctive and shared IgG and IgA-specific immune epitope signatures across the SARS-CoV-2 proteome particularly in the ORF1a/b, Spike, Membrane and Nucleocapsid phosphoprotein regions to be used as prognostic markers for the course of COVID-19 disease.

934

RAPID POINT OF CARE TESTS TO SIMULTANEOUSLY QUANTIFY ANTI-TB DRUGS IN BLOOD

Yan Zhou, Jason Zhou

Zymeron Corporation, Durham, NC, United States

Rapid Point of Care Tests to Simultaneously Quantify Anti-TB Drugs in BloodYan Zhou[a], Jason Zhou[a], Michael Liu[aa] Zymeron Corporation, Research Triangle Park, NC 27709

Tuberculosis (TB) is the world's second leading infectious killer. TB treatment includes multiple different combinations of antibiotics, doses, and long time periods. Treatment outcome for TB patients receiving multidrug therapy can be poor due to drug-drug interactions, impaired medication adherence, drug absorption and metabolism variation, and drug resistance. Therefore, therapeutic drug monitoring (TDM) remains a valuable clinical tool for using plasma drug concentrations to determine dose, correct sub-optimal drug concentrations and achieve optimal outcome. Current standard TB drug monitoring methods (i.e. LC and LC-MS) are done in highly specialized laboratories, are time-consuming and require analysis of multiple samples at the same time to reduce costs per sample. Consequently, these methods make regular monitoring of antibiotic concentrations essentially impractical. Rapid determination of antibiotic concentrations at the point of care (POC) would allow clinicians to monitor antibiotics "real-time" and allow immediate dose adjustments. This would reduce the development of resistance, treatment failure, and antibiotic induced toxicity. Zymeron has developed one-step, rapid (less than 20 minutes), low-cost, self-contained POC diagnostic tests (RapiTDM^{TM}) for quantitative measurement of multiple therapeutic drugs directly from whole blood by applying its novel nanoenhanced fluorescent lateral flow immunoassay technology. Our RapiTDM^{TM} assay overcomes the drawbacks (i.e. low sensitivity and narrow range of quantification) of conventional lateral flow assays while taking advantage of the many attributes inherent to lateral flow as an ideal rapid, low-cost, POC test that can help physicians make timely decisions to adjust the dose regimen and change treatment strategies.

935

IMPLEMENTATION OF COVID-19 TESTING IN AN ARBOVIRAL DISEASE COMMUNITY COHORT IN PONCE, PUERTO RICO

Liliana Sanchez-Gonzalez[1], Chelsea Major[1], Olga D. Lorenzi[1], Laura Adams[1], Janice Perez-Padilla[1], Dania Rodriguez[1], Kyle Ryff[1], Jorge Munoz-Jordan[1], Gilberto Santiago[1], Freddy Medina[1], Mariely Linares[2], Gladys Gonzalez-Zeno[2], Vanessa Rivera- Amill[2], Gabriela Paz-Bailey[1]

[1]*Centers for Disease Control and Prevention, San Juan, PR, United States,* [2]*Ponce Health Sciences University, Ponce, PR, United States*

Communities Organized to Prevent Arboviruses (COPA) is a community-based cohort study in southern Puerto Rico that measures the incidence of arboviral infections through annual serologic and virologic testing in 4,000 participants ages 1 to 50 years. After the first COVID-19 cases were detected in Puerto Rico in March 2020, testing for SARS-CoV-2 was implemented to detect if unrecognized community transmission was occurring. Due to the strict social distancing restrictions in place, annual follow-up interviews were conducted by phone and participants scheduled to attend a mobile clinic for sample collection. Acute febrile illness (AFI) surveillance is being implemented through an automated weekly text messaging system for the 2,250 participant households. Cohort participants and other household members reporting fever or cough during phone interviews or through AFI surveillance will also be invited to visit the clinic to provide samples. Blood samples are currently tested for dengue IgM and IgG and will be tested for anti-COVID-19 antibodies once an appropriate test is available. Symptomatic participants are currently tested for dengue, Zika, and chikungunya viruses by Trioplex Real-Time RT-PCR in serum and for SARS-CoV-2 by RT-PCR in a self-collected nasal swab. Since COVID-19 activities in COPA began, 550 participants have been interviewed by phone and 71 participants provided a blood sample. Of those, 8 participants reported fever and provided a respiratory specimen. Data collection and testing are in progress and results will be updated accordingly. Existing cohort studies can be modified to incorporate additional testing and be a useful surveillance tool during emerging public health threats, providing crucial information on community attack rates to help inform epidemic response.

936

RESPIRATORY VIRUSES ARE THE MOST COMMON CAUSE OF LOWER RESPIRATORY TRACT INFECTION IN SOUTHERN PROVINCE, SRI LANKA

Tianchen Sheng[1], Sky Vanderburg[1], Champica Bodinayake[2], Gaya Wijayaratne[2], Ajith Nagahawatte[2], Vasantha Devasiri[2], Ruvini Kurukulasooriya[2], Muhunthan Sellathurai[3], Nayomi Danthanarayana[3], Chathurangi Halloluwa[2], Kanchana Sewwandi[2], Jack G. Anderson[1], Bradly P. Nicholson[4], Christopher W. Woods[1], L. Gayani Tillekeratne[1]

[1]*Duke University, Durham, NC, United States,* [2]*Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka,* [3]*Teaching Hospital Karapitiya, Galle, Sri Lanka,* [4]*Institute for Medical Research, Durham, NC, United States*

Lower respiratory tract infection (LRTI) is the leading infectious disease-related cause of mortality worldwide and the epidemiology, etiology, and severity of LRTI is poorly understood in low- or middle-income countries. A prospective cohort study of inpatients presenting with LRTI to the largest tertiary care hospital in Southern Province, Sri Lanka, was conducted from 2018 to 2019. Patients ≥1 year old who met a case definition for LRTI were included. Nasopharyngeal samples were tested for respiratory viruses by multiplex polymerase chain reaction. Sociodemographic and clinical information were assessed using the Chi-square and Kruskall-Wallis tests. During the study period, 188 patients were enrolled. Median age was 56 years (IQR 22-69) and 327 (42.1%) were male. Overall, 96 (51.1%) had a

respiratory virus identified: influenza A 2009 H1N1 in 22 (11.7%), human rhinovirus/enterovirus (HRV/HEV) in (17, 9.0%), respiratory syncytial virus (RSV) A in 13 (6.9%), adenovirus in 12 (6.4%), bocavirus in 11 (5.8%), other influenza A types in 11 (5.8%), influenza B in 9 (4.8%), human metapneumovirus in 7 (3.7%), and RSV B in 4 (2.1%). Children <18 years of age were more likely to have RSV A (24.0% vs 3.3%, p=0.035) and bocavirus (22.0% vs 0, p=0.015) detected. Adults were more likely to have influenza A 2009 H1N1 (30.0% vs 20.0%, p=0.456) and HRV/HEV (26.7% vs 12.0%, p=0.172) detected, although these associations were not statistically significant. Of patients with a respiratory viral pathogen detected, 51 (53.1%) had chest x-rays performed and 22 (43.1%) were abnormal: opacity/consolidation in 16 (72.7%) and interstitial pattern in 4 (18.2%). Median hospitalization duration was 2 days (IQR 1-5), with patients with a respiratory virus detected having a longer stay (median 3 days versus 1 day, p=0.013). Overall, 3 (4.2%) patients died, with 1 death (1.4%) in a patient with a respiratory virus detected. Respiratory viruses were the most common etiology of LRTI in this cohort, but the majority of patients were treated with antibiotics. Improved diagnostics and further epidemiologic studies are essential for improving the management of LRTI in this setting.

937

OXYGEN MANAGEMENT FOR CHILDREN DYING FROM PNEUMONIA IN KENYA: FINDINGS FROM THE CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE (CHAMPS)

Jennifer R. Verani[1], Victor Akelo[2], Dianna M. Blau[3], Aggrey Igunza[4], Magdalene Kuria[5], Florence Murila[6], Emily A. Rogena[7], Gunturu Revathi[8], Paul Mitei[9], Benard Ogony[4], Elizabeth Oele[10], Samuel Omondi[11], Emily Zielinski-Gutierrez[12], Pratima Raghunathan[1], Marc-Alain Widdonson[12], Cynthia G. Whitney[13], Dickens Onyango[10], Beth Barr[2], Robert F. Breiman[13]

[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 and Emory University, Emory Global Health Institute, Atlanta, GA, United States,* [4]*Kenya Medical Research Institute, Kisumu, Kenya,* [5]*Jaramogi Oginga Odinga Teaching and Referral Hospital, Kisumu, Kenya,* [6]*University of Nairobi, Nairobi, Kenya,* [7]*School of Medicine, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya,* [8]*Aka Khan University, Department of Microbiology, Nairobi, Kenya,* [9]*Kisumu Specialists Hospital, Kisumu, Kenya,* [10]*Kisumu County Department of Health, Kisumu, Kenya,* [11]*Siaya County Department of Health, Siaya, Kenya,* [12]*Centers for Disease Control and Prevention, Nairobi, Kenya,* [13]*Emory University, Emory Global Health Institute, Atlanta, GA, United States*

Hypoxemia is an important marker of pneumonia severity, but often overlooked or inadequately managed in resource-poor settings. We examined hypoxemia detection and oxygen delivery among fatal pediatric pneumonia cases in Kenya. We analyzed data from Child Health and Mortality Prevention Surveillance (CHAMPS), which identifies deaths among children aged <5 years in an urban and rural area in western Kenya. Parents of deceased are interviewed, medical records reviewed and postmortem samples collected and tested. An expert panel determines immediate, morbid, and underlying causes of death. From May 2017 to July 2019, 37/213 (17.4%) enrolled deaths occurring in health facilities (35 in hospitals and 2 in health center/post) had pneumonia in the causal chain of death. The median age among children with pneumonia in the causal chain was 7.0 months (interquartile range [IQR] 1.4-12.7). Eighteen (48.6%) had documented oxygen saturation (SpO2); median lowest SpO2 was 72.5% (IQR 45.0-84.0), and 16 (88.9%) were <90%. Among 35 with SpO2<90% or missing, 23 (65.7%) had data on oxygen management; 21 (91.3%) received oxygen, including 12 (57.1%) by nasal cannula only, 5 (23.8%) nasal cannula and facemask, 2 (9.5%) facemask only, and 2 (9.5%) not specified. We observed gaps in SpO2 measurement and suboptimal use of oxygen that may contribute to child pneumonia deaths in Kenya. Efforts are needed to improve SpO2 measurement and documentation, and to optimize oxygen delivery for children with pneumonia and hypoxemia.

938

ADHERENCE TO TUBERCULOSIS PREVENTIVE THERAPY IN A NOVEL COMMUNITY-BASED DIFFERENTIATED CONTACT MANAGEMENT PROGRAM IN ESWATINI

Micaela Sandoval[1], Alexander W. Kay[2], Martha Matsenjwa[3], Godwin Mtetwa[3], Tara Devezin[2], Gloria Sisi Dube[3], Joyce Sibanda[3], Welile Sikhondze[4], Bhekumusa Lukhele[3], Anna Maria Mandalakas[2]

[1]*UTHealth School of Public Health, Houston, TX, United States,* [2]*The Global TB Program, Texas Children's Hospital, Immigrant and Global Health, Baylor College of Medicine, Houston, TX, United States,* [3]*Baylor College of Medicine Children's Foundation, Mbabane, Swaziland,* [4]*National TB Control Program, Ministry of Health, Swaziland, Mbabane, Swaziland*

In April 2019, we implemented Vikela Ekhaya, a large-scale community-based TB contact management program in Eswatini, formerly Swaziland. Eswatini carries a high TB/HIV burden; over 70% of TB cases are HIV-positive, and the prevention of TB disease in children exposed to TB is a public health priority. Vikela Ekhaya offered differentiated TB and HIV testing and care for household contacts of TB cases, by utilizing mobile contact management teams to screen contacts, assess their eligibility for new, shorter, rifampicin-based TB preventive therapy (TPT) regimens, and monitor TPT adherence in participants' homes. By April 2020, 962 contacts from 247 households were offered community or facility-based evaluations. Of those screened, 97% of the 331 eligible asymptomatic household contacts (80% children under 5) from 157 families initiated TPT and were followed for the duration of their therapy. 247 children under 15 initiated 3HR, a 3 month child-friendly, dispersible rifampicin-based regimens, while 73 adults and children living with HIV initiated 6H, a 6 month isoniazid tablet regimen (standard of care). Self-reported adherence to TPT regiments was regularly monitored in TB clinics or in participants' homes by the mobile contact management teams. Pill counts, self-reported missed doses, and qualitative measures of drug acceptability, ease of administration, and caregiver knowledge, attitudes, and beliefs about TPT and latent tuberculosis infection were collected to supplement adherence monitoring. Here, we present a robust, nuanced analysis of multiple measures of adherence to TB preventative therapy among high-risk household contacts of TB cases in a high burden/ low resource setting. We observed a crude adherence rate of 96% across all regimens and care modalities, with 6H regimen, facility-based care, and monthly household income under E1000 emerging as risk factors for discontinuing treatment in time to event models. The findings of our programmatic evaluation will inform public health policy and support the development of differentiated care strategies for household contacts of TB cases, especially children, in endemic areas.

939

HIGH POLYPARASITISM BURDEN IN EL SALVADORIAN CHILDREN: *TRYPANOSOMA CRUZI* AND GASTROINTESTINAL PARASITES

Melissa S. Nolan[1], Mary K. Lynn[1], Stanley Rodriguez[2], William E. Murcia[3], Maria J. Villar-Mondragon[4], Rojelio Mejia[4]

[1]*University of South Carolina, Columbia, SC, United States,* [2]*University of El Salvador, San Salvador, El Salvador,* [3]*El Salvador Ministry of Health-Western Region (SIBASI), Sonzacate, El Salvador,* [4]*Baylor College of Medicine, Houston, TX, United States*

Despite the global commonality of both *Trypanosoma cruzi* infection a.k.a. Chagas disease (CD; 6+ million infected)) and gastrointestinal (GI; 795+ million infected) parasites, the pathophysiology of co-infection is unknown. Both CD and GI infections cause significant chronic morbidity, and an understanding of co-infected health outcomes is critical to identify targets for early clinical detection and effective therapeutic regimens. Our pilot study in the western region of El Salvador performed the first CD and GI surveillance in over twenty years, and 168 children participated in both GI fecal and CD blood testing. GI parasites and CD were common: 75% and 1.2%, respectively. Interestingly, *Ascaris* and *Strongyloides* were not present in our cohort; however, *Necator*, *Giardia*, *Blastocystis*, and *Trichuris*

were recorded. Polyparasitism was evident in 14% of the pediatric cohort and was significantly associated (p<0.002) with increased levels of *Giardia* and *Blastocystis* parasites. The findings of our study support a growing body of literature suggesting that polyparasitism results in increased parasite levels which could limit the efficacy of mass drug administration campaigns.

940

PREVALENCE AND PHYLOGENETIC ANALYSIS OF *TOXOPLASMA GONDII* OF DOMESTIC AND STRAY DOGS AND CATS IN KOREA FROM 2016-2017

Yun Sang Cho, Yeojin Park, Jinhyeong Noh, Hyun-Ji Seo, Keun-Ho Kim, Subin Min, Mi-Sun Yoo, Bo-Ram Yun, Jong-Ho Kim, Eun-Jin Choi, Doo-Sung Cheon, Sung-Jong Hong, Soon-Seek Yoon

Animal and Plant Quarantine Agency, Gimcheon, Republic of Korea

Human toxoplasmosis is a zoonotic infectious disease that is asymptomatic in the majority of infected people but can cause serious diseases or even be fatal in immunocompromised patients, elderly, and pregnant women. The outbreak of human toxoplasmosis can be attributed to ingestion of food contaminated with the *Toxoplasma gondii* pathogen. Recently, the increase in both domestic and stray dogs and cats has prompted studies on the zoonotic infectious diseases that are transmissible via these animals. In this study, toxoplasmosis ELISA antibody titers were measured on sera of 403 stray cats, 947 stray dogs, 909 domestic cats, and 2,412 domestic dogs collected from various regions in Korea from 2016 to 2017. In addition, whole blood, feces, and tissue samples were also collected from stray cats (1,392), stray dogs (686), domestic cats (3,040), and domestic dogs (1,974), and *T. gondii*-specific B1 gene PCR was performed. The seroprevalence of stray cats and stray dogs, domestic cats, and domestic dogs were 14.14%, 5.60%, 2.31%, and 0.04%, respectively. In addition, the antigen prevalence of these animals was 0.50%, 0.15%, 0.10%, and 0.35%, respectively. Moreover, stray cats had the highest infection rate of toxoplasmosis, followed by stray dogs, domestic cats, and domestic dogs. These findings highlight that strengthening efforts as the One Health level related to hygienic prevention management in dogs and cats that have frequent contact with humans, as well as those exposed to the environment, to minimize the rate of human toxoplasmosis.

941

DEVELOPMENT OF A LOOP-MEDIATED ISOTHERMAL AMPLIFICATION ASSAY (LAMP) TO SPECIFICALLY DETECT *TRICHOMONAS TENAX*

Maurice A. Matthew, Chaoqun Yao

Ross University School of Veterinary Medicine, Basseterre, Saint Kitts and Nevis

Abstract: Development of a Loop-Mediated Isothermal Amplification Assay (LAMP) to specifically detect *Trichomonas tenax*.Maurice A. Matthew and Chaoqun YaoRoss University School of Veterinary Medicine and One Health Center for Zoonoses and Tropical Veterinary Medicine, St. Kitts & Nevis, West Indies.

Periodontal disease is a widespread disease affecting both humans and animals worldwide. Over the years, the disease has become one of the most prevalent diseases globally and raised public health concerns. *Trichomonas tenax*, a flagellated protozoan usually found in the oral cavity of humans and dogs has been associated with periodontal disease. Its prevalence in humans and dogs can range from 4% to 53% and 15% to 25%, respectively. A diagnostic method of high specificity, sensitivity, simplicity, and yet at a low cost could be paramount in controlling the widespread of this parasite. Loop-Mediated Isothermal Amplification (LAMP) has been widely applied in the detection of infectious viral, bacterial and parasitic diseases. In this study, a LAMP assay was developed to detect *T. tenax*. Six primers were designed using the Primer Explorer software, targeting the ITS and 5.8S rRNA gene of this trichomonad protozoan. The LAMP procedure was optimized for primer ratio, magnesium concentration, and operating temperature. It was further contrast to a similarly optimized PCR method targeting the same DNA fragment. Results were visualized and analyzed using both SYBR Safe and gel electrophoresis. Its limit of detection was determined and specificity was confirmed using DNA of several bacterial and parasitic organisms. By developing this LAMP assay, *T. tenax* can be rapidly detected with high specificity and sensitivity in both human and canine patients with suspected periodontal disease to help control the spread of the disease worldwide. This LAMP assay could be a rapid, simple, low-cost and specific test used at the point of care for clinical diagnosis.

942

EXPLORATION OF MOLECULAR DETECTION TECHNIQUES USED TO DIAGNOSE *CYCLOSPORA CAYETANENSIS*

Alexandria Albano, Ana Sanchez

Brock University, St. Catharines, ON, Canada

Diarrheal disease is one of the leading causes of global morbidity and mortality associated with ~1.3 million deaths annually. Diarrheal disease is usually a symptom of an infection in the small intestinal tract, which may be caused by a variety of bacterial, viral, and parasitic organisms. A significant cause of diarrheal disease is through contaminated food and/or water. Due to the increase in the globalization of food supply, consumption of fresh foods, and international travel, the risk of exposure to the developed world to uncommon tropical diseases has also increased. *Cyclospora cayetanensis* is an intestinal protozoan parasite that causes diarrheal disease in those who are infected. *C. cayetanensis* is obtained through the consumption of contaminated fresh foods and/or water with infective oocysts. Recently, there has been an increase in the amount of cyclosporiasis cases in North America associated with the consumption of fresh foods. Diagnosis of human cyclosporiasis is typically performed on collected stool samples to identify *C. cayetanensis* oocysts using well established parasitological techniques including a variety of differential staining techniques, such as safranin staining, and microscopy techniques, such as bright-field microscopy and epifluorescence. In terms of molecular detection assays, there has been limited research focused on the development of this technique. Currently, there are few molecular detection assays that have been used for genetic analysis, detection of mutations, and genetic categorization of *C. cayetanensis*. However, these techniques are not currently used as routine diagnostic techniques as they have not been optimized for diagnostic use. The following study aims to optimize a nested PCR protocol that can be used in the diagnosis of cyclosporiasis using samples collected from asymptomatic children living in areas of Honduras where the prevalence for cyclosporiasis was previously established. Furthermore, a comparison between the nested PCR protocol and epifluorescence will be conduction to determine which technique is more sensitive and specific for diagnosing human cyclosporiasis.

943

PREVALENCE AND GENOTYPING OF *TOXOPLASMA GONDII* IN PREGNANT WOMEN ATTENDING THE ARISTIDE LE DANTEC UNIVERSITY HOSPITAL IN DAKAR, SENEGAL

Ibrahima Mbaye Ndiaye

Cheikh Anta Diop University of Dakar, Dakar, Senegal

Toxoplasma gondii is one of the most widespread parasites in the world that infects humans and other warm-blooded animals. It causes asymptomatic toxoplasmosis in immunocompetent adults, while in pregnant women primary infection can lead to congenital toxoplasmosis in fetuses and newborns. Therefore, in pregnant women, early and accurate toxoplasmosis status screening can be crucial for prevention and control of the disease. Routinely, immune status against *T. gondii* is assessed by identifying parasite-specific antibodies in the serum with serological techniques such as Enzyme-linked Immunosorbent Assay. Another factor of the *T. gondii* infection severity in humans is the strain virulence of the parasite which is non-documented in Senegal. Three genetically different types (strains) are described (type I, type II and type III), based on the

genetic analysis of the polymorphic surface antigen 2 locus (SAG2) using a PCR-RFLP method. Congenital toxoplasmosis is associated mainly with type I and II strains. Thus, identification of the genetic type helps to better understand the disease, and possibly help to find the appropriate treatment. Hence the aim of this study was to determine the lineage types of *T. gondii* in pregnant women diagnosed with positive serology against *T. gondii* in Senegal. From January to December 2016, 104 pregnant women attending the Parasitology-Mycology laboratory of Le Dantec University Hospital, Dakar were enrolled. Among them, 48 (46.2%) were found with IgG antibodies. Genetic assessment using the B1 gene-PCR, targeting the repetitive 35-fold B1 gene, of these samples revealed 20 positive cases (41.7%), confirmed by 5'-3' SAG2 gene-PCR. Enzymatic digestion of positive samples successively with the HhaI and the Sau3AI enzymes revealed 85% of these positive samples represented type III infections, and 15% represented type I infections. This study represents the first analysis of *T. gondii* infections among pregnant women in Senegal, and revealed that type III of *T. gondii* was predominant, while none of the infections were of the type II strain.

945

TRANSCRIPTOMIC CHARACTERIZATION OF THE EARLY HOST RESPONSE TO *BABESIA ROSSI* INFECTION

Rachel L. Smith[1], Amelia Goddard[2], Johan Schoeman[2], Arun Boddapati[3], Steven Brooks[1], Justin Lack[3], Andrew Leisewitz[2], Hans Ackerman[1]

[1]*Laboratory of Malaria and Vector Research, NIAID, Rockville, MD, United States,* [2]*Faculty of Veterinary Science, University of Pretoria, Onderstepoort, Pretoria, South Africa,* [3]*Collaborative Bioinformatics Resource, NIAID, Frederick, MD, United States*

Babesia rossi (*B. rossi*) is the primary agent of canine babesiosis in South Africa. In order to gain insight into the early host response to *B. rossi* infection, we analyzed the whole blood transcriptome at baseline and one day after inoculation. Three canines were inoculated with 10^9 *B. rossi* parasites and two were infected with 10^4 parasites on an experimental protocol approved by the University of Pretoria Animal Ethics Committee (V003-18). RNA was extracted from whole blood drawn on days 0 and 1. PolyA-selected mRNA libraries were generated and all samples were pooled and sequenced on a single S1 flowcell on the Illumina NovaSeq machine. Reads were mapped to the canine reference genome, and genes with fewer than 20 raw reads were excluded. Differential gene expression was carried out using DESeq2 for the day 1 versus day 0 comparison. Genes with absolute fold change greater than 1.5 and adjusted p-value less than 0.05 were subjected to gene set enrichment analysis using Gene Ontology (GO). In the high-dose group, 240 genes were upregulated and 48 genes were downregulated on day 1; whereas in the low-dose group, no genes were significantly differentially expressed. GO enrichment analysis indicated that many of the upregulated genes were involved in the innate immune response, while many of the downregulated genes were associated with the adaptive immune response. Interestingly, cellular gene pathways typically associated with viral infection, such as type I interferons, were significantly upregulated, while pathways associated with bacterial infection, such as natural killer cell chemotaxis, were downregulated. In conclusion, we report a panel of 288 genes significantly changed within the first 24 hours of a high-dose *B. rossi* infection. The dose-dependent activation of these genes in response to the onset of parasitemia revealed activation of specific pathways involved in innate immunity, counterbalanced by induction of immune regulatory genes.

946

MAPPING AND GEOGRAPHIC DISTRIBUTION OF *BURKHOLDERIA PSEUDOMALLEI* IN MYANMAR

Myo Maung Maung Swe[1], Mo Mo Win[2], Joshua Cohen[1], Aung Pyae Phyo[1], Daniel Parker[3], David Dance[4], Elizabeth Ashley[4], Frank Smithuis[1]

[1]*Myanmar Oxford Clinical Research Unit, Yangon, Myanmar,* [2]*Department of Medical Research, Ministry of Health and Sports, Yangon, Myanmar,* [3]*Department of Population Health and Disease Prevention Program in Public Health, University of California, Irvine, CA, United States,* [4]*Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit, Vientiane, Lao People's Democratic Republic*

Melioidosis is a serious tropical infection first described in 1912 in Yangon General hospital in Myanmar. It is caused by a gram negative bacterium called *Burkholderia pseudomallei* found in the soil. Although it is a common cause of sepsis in neighbouring countries, it is rarely diagnosed nowadays in Myanmar. We conducted a nationwide soil study to identify areas where *B.* pseudomallei is present in the environment. Sampling sites were selected using a geographic information system across all 15 regions of Myanmar between September 2017 and June 2019. At each site, 9 samples were taken, 5 meters apart at three different depths: 30, 60 and 90 cm. In addition 1 pooled sample was prepared. All samples were cultured within 48 hours to isolate *B. pseudomallei*. 3870 soils samples were cultured from 387 sites. 8% (31/387) of sites had one or more positive samples and 2.7% (103/387) of samples tested positive for *B. pseudomallei*. *B. pseudomallei* was isolated in 7 of 15 regions. The isolation rate was higher in the rainy season (5%; 57/1140) compared to the cool season (2%; 31/1550) and the hot season (1.3%: 15/1180). The depth of the sample was not associated with isolation of *B. pseudomallei*; 2.4% (28/1161) each for 30 cm and 60 cm and 2.6% (17/1161) for 90 cm depths. In terms of land use, samples taken from pastureland had a higher rate of isolation (8.5%; 11/130) than unused land (5.8%; 21/360), rice/cultivated fields (2.3%; 70/3090) and residential areas (0.5%; 1/200) respectively. This first nationwide study demonstrates a widespread distribution of *B. pseudomallei* in the soil of Myanmar. Further clinical studies should follow in regions where *B. pseudomallei* was isolated. Clinicians need to be aware of melioidosis as a potential cause of sepsis and pneumonia and other presentations in order to facilitate early diagnosis and treatment.

947

DEVELOPMENT AND OPTIMIZATION OF A RAPID TEST FOR TRACHOMA ELIMINATION PROGRAMS

Sarah Gwyn[1], Scott Nash[2], E. Kelly Callahan[2], Andrew W. Nute[2], Tigist Astale[2], Ambahun Chernet[2], Eshetu Sata[2], Mulat Zerihun[2], Zerihun Tadesse[2], Danaya Bethea[1], Christian Laurent[1], Diana Martin[1]

[1]*CDC, Atlanta, GA, United States,* [2]*The Carter Center, Atlanta, GA, United States*

Trachoma is the leading infectious cause of blindness and is targeted for elimination as a public health problem by 2030. Because the elimination threshold (less than 5% trachomatous inflammation—follicular (TF) in children ages 1-9-years) still allows the possibility of low-level transmission, a test capable of detecting recrudescence of infection will be necessary once countries achieve elimination. We have developed several tests to detect antibodies against the *Chlamydia trachomatis* antigen Pgp3, including a multiplex bead assay (MBA) and a lateral flow assay (LFA). The LFA is subject to reader-to-reader variability. We evaluated the performance of these tests in two districts in the Amhara region of Ethiopia: one with high TF (Andabet, TF = 37.7% [95% confidence interval [CI]: 31.1-43.3], N = 708) and one with low TF (Alefa, TF = 3.2% (95% CI 1.4-5.7], N = 583). Dried blood spot specimens were collected from 1-9-year-olds during trachoma impact and surveillance surveys in 2017 and were tested on the MBA and two versions of the LFA: the original version using a colloidal gold detection reagent (LFA-gold) and a newer version using

a black latex detection reagent (LFA-latex). Tests run on LFA-latex were read by two readers to assess inter-rater agreement. Seroprevalence for Andabet was 37.0% (95% CI 33.2-41.0) by MBA, 37.7% (95% CI 33.9-41.7) by LFA-gold and 42.5 (95% CI 38.6-46.6) by LFA-latex. Inter-rater agreement for LFA-latex was κ = 0.986 (95% CI 0.97-1.00; this was not estimated for LFA-gold). Seroprevalence for Alefa was 1.4% (95% CI 0.7-2.6) by MBA, 9.8% (95% CI 7.8-12.2) by LFA-gold and 2.8 (95% CI 1.8-4.4) by LFA-latex. Inter-rater agreement for LFA-gold using Cohen's kappa (κ) was 0.631 (0.50-0.76) and by LFA-latex was 1.000 (95% CI 1.00-1.00). These data suggest that the LFA-latex test is more robust than LFA-gold, providing comparable population-level seroprevalence data as MBA, excellent percent agreement with the MBA, and high inter-rater agreement, particularly in low-prevalence settings.

948

GENOMICS OF OCULAR *CHLAMYDIA TRACHOMATIS* AFTER FIVE YEARS OF SAFE INTERVENTIONS FOR TRACHOMA IN AMHARA, ETHIOPIA

Harry Pickering[1], Ambahun Chernet[2], Eshetu Sata[2], Mulat Zerihun[2], Charlotte A. Williams[3], Judith Breuer[3], Andrew W. Nute[4], Mahiteme Haile[5], Taye Zeru[5], Zerihun Tadesse[2], Robin L. Bailey[1], E. Kelly Callahan[4], Scott D. Nash[4], Martin J. Holland[1]

[1]*Department of Clinical Research, London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*The Carter Center, Addis Ababa, Ethiopia,* [3]*Division of Infection and Immunity, University College London, London, United Kingdom,* [4]*The Carter Center, Atlanta, GA, United States,* [5]*Amhara Public Health Institute, Bahir Dar, Ethiopia*

To eliminate trachoma as a public health problem, the WHO recommends the SAFE (Surgery, Antibiotics, Facial cleanliness, and Environmental improvement) strategy. As part of the SAFE strategy in the Amhara Region, Ethiopia, the Trachoma Control Program distributed over 124 million doses of antibiotics between 2007 and 2015. The program also provided village- and school-based health education as well as assisted in the construction of latrines throughout the region as part of the F and E components. Despite an average of 5 years of these interventions, trachoma remained hyperendemic in many districts as measured by the indicator trachomatous inflammation-follicular (TF), and a considerable level of *Chlamydia trachomatis* (*Ct)* infection was evident region-wide. This study utilized residual material from m2000 Abbott tubes used in previous infection assays, to sequence 99 ocular *Ct* samples from Amhara and investigate the role of genomics in the continued transmission of *Ct* following 5 years of SAFE. These whole-genome sequences were further utilized to explore the relationship between *Ct* genomic variation and infection and trachoma prevalence at village and district level. Sequences were typical of ocular *Ct*, at both the whole-genome level and in tropism-associated genes. There was no evidence of macrolide-resistance alleles in this *Ct* population. Polymorphism in a region around the serovar-determining *ompA* gene was associated with village-level TF prevalence. Additionally, the presence of multiple *ompA* serovars in a village and greater *ompA* diversity at the district-level were both associated with increased *Ct* infection prevalence. Our data found no evidence for *Ct* genomic variation contributing to continued transmission of *Ct* after multiple rounds of treatment, adding to previous evidence that azithromycin does not drive the acquisition of macrolide resistance in *Ct*. The finding of higher *Ct* infection in villages harboring multiple *ompA* serovars, as well as in districts with greater *ompA* diversity, require longitudinal investigation to understand what impact this may have on treatment success and development of host immunity.

950

TRACHOMA ELIMINATION CHALLENGES: INVESTIGATING REASONS FOR RECRUDESCENCE OF TRACHOMA IN FOUR DISTRICTS OF MOZAMBIQUE

Marilia Massangaie[1], Mariamo Abdala[1], Mawo Fall[2], Tamimo Momade[2], Zulficar Bay[2], Hannah Frawley[3], Sabrina Eyob[3], Scott McPherson[4], Jeremiah M. Ngondi[3]

[1]*Ministry of Health, Maputo, Mozambique,* [2]*RTI International, Maputo, Mozambique,* [3]*RTI International, Washington,, DC, United States,* [4]*RTI International, RTP, NC, United States*

Mozambique started implementation of the surgery, antibiotics, facial cleanliness and environmental change (SAFE) strategy for trachoma elimination in 2011. Of the 65 districts classified as endemic at baseline, 33 (51%) have attained the World Health Organization (WHO) elimination threshold for trachomatous inflammation-follicular (TF) in children aged 1-9 years of <5%. The WHO recommends trachoma surveillance survey (TSS) at least 24 months after trachoma impact surveys (TIS) have showed that TF was <5%. We investigated the reasons for recrudescence of TF in four districts where TSS showed TF was >5%. Data on mass drug administration (MDA), survey history, and household access to water hygiene and sanitation (WASH) were reviewed. MDA data showed that three districts (baseline TF category=10.0-29.9%) had received only 2 of 3 recommended MDA rounds and TIS was done to investigate if two rounds (instead of three) were adequate to attain elimination of TF. The fourth district (baseline TF category=5.0-9.9%) has received the recommended one round of MDA. Reported MDA data showed that coverage was sufficient (≥80%) for all MDA rounds in the four districts. TSS showed lower household access to water facilities (4-35%, range by district) and sanitation facilities (1-9%) across all districts compared to the national average of 49% and 24%, respectively. Recrudescence of TF is a potential draw-back to attainment of elimination of trachoma as a public health problem. Therefore, in addition to undertaking high quality and high coverage MDA, TIS should be done when the recommended number of MDA rounds is attained and increased advocacy for access to WASH is needed to minimize transmission of trachoma.

951

THE PREVALENCE OF TRACHOMA IN TREATMENT-NAÏVE NORTH DARFUR REGION: RESULTS FROM POPULATION-BASED BASELINE SURVEYS, NORTH DARFUR, SUDAN

Zeinab Abdalla[1], Angelia M. Sanders[2], Balgesa E. Elshafie[3], Atif E. Mohammedsalih[1], Andrew W. Nute[2], Nabil Aziz[1], E. Kelly Callahan[2], Scott D. Nash[2]

[1]*The Carter Center, Khartoum, Sudan,* [2]*The Carter Center, Atlanta, GA, United States,* [3]*Sudan Ministry of Health, Khartoum, Sudan*

A global effort in trachoma mapping has resulted in few places remaining in the world where the trachoma burden is not known. With improved access and greater collaboration with local governments, Darfur has become available for trachoma baseline mapping and the subsequent programmatic activities needed to eliminate trachoma as a public health problem. In 2019-2020 the Sudan Trachoma Control Program conducted population-based trachoma surveys in 3 localities (districts) in North Darfur state, Sudan. Multi-stage cluster random sampling was used to select 30 villages (clusters) of 25 households per locality. In addition to the collection of trachoma clinical data by trained and certified trachoma graders, trained nurses collected dried blood spot (DBS) samples from individuals within the selected households. 8,325 individuals aged ≥1 year in 2,189 households across the 3 localities were examined for trachoma clinical signs. The prevalence of trachomatous inflammation-follicular (TF) among children aged 1-9 years was 1.4% (95% Confidence interval [CI]: 0.8-2.7) in Kotom, 11.0% (CI: 7.6-15.7) in Seraf Omrah, and 15.6% (CI: 10.9-21.7) in El Seraif localities. Trachomatous inflammation-intense (TI) was <0.5% for all 3 localities, which were above the trachomatous trichiasis (TT) elimination target of <0.2% TT in those aged ≥15 years. Household latrine ownership ranged between 31-63%, and clean face

among children 1-9 years ranged between 64-76%. Results showed that 2 of the localities require 3 rounds of annual mass drug administration with azithromycin and all 3 localities will require TT surgical campaigns and health education regarding latrine use and personal hygiene. DBS samples will be assayed for antibody responses to multiple antigens as a potential measure of population-level exposure to ocular *Chlamydia trachomatis*. Globally and within Sudan, post-conflict regions will be the last remaining areas needing interventions. As localities become accessible in Darfur, Sudan continues to complete their baseline trachoma mapping and thus moves closer to trachoma elimination as a public health problem.

952

TRACHOMA, OCULAR *CHLAMYDIA TRACHOMATIS* INFECTION, AND ANTI-PGP3 SEROLOGY AMONGST CHILDREN IN THE REPUBLIC OF NAURU

Kathleen D. Lynch[1], Sue Chen Apadinuwe[2], Tessa Hillgrove[3], Stephen B. Lambert[4], Anasaini T. Cama[3], Sara Webster[3], Mitchell Starr[5], Beth Catlett[5], Emma Harding-Esch[6], Ana Bakhtiari[7], Robert Butcher[8], Chandalene Garabwan[2], Anthony W. Solomon[9], John M. Kaldor[10], **Susana Vaz Nery**[11]

[1]*Child Health Research Centre, The University of Queensland, Brisbane, Australia,* [2]*Ministry of Health and Medical Services, Denig, Nauru,* [3]*The Fred Hollows Foundation, Melbourne, Australia,* [4]*The University of Queensland, Brisbane, Australia,* [5]*NSW State Reference Laboratory for HIV, St Vincent's Hospital Sydney, Sydney, Australia,* [6]*London School of Hygiene &Tropical Medicine, London, United Kingdom,* [7]*The Task Force for Global Health, Decatur, GA, United States,* [8]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [9]*Department of Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland,* [10]*The Kirby Institute, UNSW, Sydney, Australia,* [11]*The Kirby Institute, UNSW, Kensignton, Australia*

Trachoma, caused by ocular *Chlamydia trachomatis* (C*t*) strains, is the leading infectious cause of blindness worldwide. There is a global commitment to eliminate trachoma as a public health problem through the implementation of the "SAFE" strategy: surgery for trichiasis, antibiotics to clear infection, and facial cleanliness and environmental improvement to reduce transmission. The need for these interventions is based on prevalence of clinical signs; countries have therefore undertaken prevalence surveys. In several Pacific countries where surveys have been conducted, a high prevalence of trachomatous inflammation—follicular (TF) in children aged 1-9 years is not always accompanied by high levels of trachomatous trichiasis (TT) in adults >15 years, and indeed much of the TF in those settings may not be due to the presence of *Ct* infection. In July 2019, the Nauru Ministry of Health and Medical Services led a survey using the WHO-recommended methodologies to determine the prevalence of trachoma and trichiasis in the country. This survey was accompanied by an investigation of the presence of ocular *Ct* infection and previous *Ct* exposure. Ocular swabs and dried blood spots (DBS) were collected from children aged 1-9 years. Nucleic acid was extracted from the swabs and amplified via real-time PCR. The DBS were used to measure antibodies against immunodominant *Ct* antigen PgP3 by ELISA and by lateral flow-based assay (LFA). Preliminary analysis of the data is as follows. The age-adjusted prevalence of TF in the 818 children examined was 22%, *Ct* infection prevalence was 34.9% (272/780), and 34.8% of children (276/792) were seropositive for anti-Pgp3 antibodies. Both *Ct* infection and seropositivity were significantly associated with TF (Pearson's X^2 p=<0.001), and seropositivity was also associated with *Ct* infection. The risk of being seropositive significantly increased for each year of increasing age (OR=1.24 95% CI 1.17-1.33 p=<0.001). Based on these results, Nauru will implement the SAFE strategy for elimination of trachoma as a public health problem.

953

PLASMODIUM FALCIPARUM GROWTH IN ERYTHROCYTES IS GOVERNED BY HEMOGLOBIN GENOTYPE AND ENDOGENOUS EXOSOMAL MICRORNA LET-7I-5P

Keri Oxendine Harp[1], Daniel Addo-Gyan[2], Felix Botchway[3], Yvonne Dei-Adomakoh[4], Michael D. Wilson[2], Andrew A. Adjei[3], Jonathan K. Stiles[1], Adel Driss[1]

[1]*Morehouse School of Medicine, Atlanta, GA, United States,* [2]*Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana,* [3]*Korle-Bu Teaching Hospital, University of Ghana Medical School, Accra, Ghana,* [4]*Korle Bu Teaching Hospital, Accra, Ghana*

Despite global reductions in malaria cases, 228 million cases and 405,000 deaths occurred in 2018 in regions of intense malaria transmission that overlap regions where sickle-cell disease (SCD) also occurs. SCD is a severe hereditary form of anemia in which hemoglobin (Hb) is mutated (HbSS/HbSC) causing red blood cells (RBC) to distort into a crescent shape at low oxygen levels. Sickle cell trait individuals inherit one mutated allele variant (HbAS/HbAC) and have a reduced risk of severe malaria compared to HbAA controls. Previous *in-vitro* studies determined that microRNAs (miRNAs), like let-7i-5p, are differentially expressed in erythrocytes derived from individuals with different Hb genotypes and correlated with parasite growth rates. We tested the hypothesis that *Plasmodium* infection rates is mediated by RBC's Hb genotypes and exosomal let-7i-5p expression. We conducted a cross sectional study in Accra, Ghana as part of two ongoing NIH funded studies. Blood samples were obtained from uninfected individuals of matching ages and sexes who visited the hematology laboratory at the Korle Bu Teaching Hospital and had different Hb genotypes (HbAA, HbAS, HbAC, HbSS, HbSC, HbCC). Pregnant, HIV, and malaria positive individuals were excluded. RBC from each Hb genotype were infected with *Plasmodium falciparum* 3D7 to a final parasitemia of 1% *in vitro*. Parasite growth rate was counted daily using Giemsa stained smears and supernatants collected over a period of 16-days to obtain exosomes. RNA was extracted from exosomes and let-7i-5p levels were determined using RT-qPCR. Observed growth patterns of parasites remained essentially the same, but growth rates differed significantly ($p<0.05$) between the different Hb genotypes. Exosomal let-7i-5p levels fluctuated between Hb genotypes and correlated ($P<0.01$, $R^2=0.88$) with parasite counts. We conclude that exosomal let-7i-5p levels and Hb genotype mediate erythrocyte parasite growth rates. Understanding the combined effects of exosomal let-7i-5p and Hb genotype on *Plasmodium* growth could lead to identification and development of novel malaria therapeutics.

954

VARIATIONS WITHIN *NFκBIA AND NFκB1* PROMOTERS PREDICT LONGITUDINAL SUSCEPTIBILITY TO PEDIATRIC MALARIAL ANEMIA AND REDUCED ALL-CAUSE MORTALITY IN KENYA

Elly Munde[1], Samuel B. Anyona[2], Evans Raballah[3], Benjamin McMahon[4], Nick Hengartner[4], Kristan A. Schneider[5], Clinton Onyango[6], Ivy Hurwitz[6], Qiuying Cheng[6], Christophe G. Lambert[6], Collins Ouma[7], Douglas J. Perkins[6]

[1]*Kirinyaga University School of Health Science, Kerugoya, Kenya,* [2]*Maseno University School of Medicine, Maseno, Kenya,* [3]*Masinde Muliro University of Science and Technology, Kakamega, Kenya,* [4]*Theoretical Biology and Biophysics Group, Theoretical Division, Los Alamos National Laboratory, Los Alamos, NM, United States,* [5]*Department Applied Computer and Bio Sciences, University of Applied Sciences Mittweida, Mittweida, Germany,* [6]*Center for Global Health, Department of Internal Medicine, University of New Mexico, Albuquerque, NM, United States,* [7]*Maseno University School of Public Health and Community Development, Maseno, Kenya*

Currently, the molecular mechanisms that culminate into pediatric severe malaria anemia (SMA, Hb<5.0g/dL) are not fully deciphered. Analysis of inflammatory pathways leading to SMA may aid in understanding the pathogenesis. Whilst genetic variations in *NFκB1* and *NFκBIA*

genes responsible for transcription regulation have been linked to pathogenesis of inflammatory diseases, their role in SMA remains unknown. We hypothesized that polymorphisms within promoters of *NFκB1* and *NFκBIA* may impact on clinical outcomes of pediatric malaria during the development of clinical immunity. Influence of *NFκB1* SNPs [rs747559 (-8079G/A) and rs980455 (-3297C/T)] and *NFκBIA* variants [rs2233406 (-826G/A), and rs2233409 (-310G/A)] on cross-sectional and longitudinal outcomes (36 mons follow-up) were determined in children (n=1,271, aged ≥3 mons) from Siaya County, western Kenya, a *P. falciparum* holoendemic transmission area. Cross-sectional bivariate regression analyses controlling for anemia covariates, revealed that carriage of *NFκBIA* -310 GA had increased malaria susceptibility (OR=1.56, 95%CI=1.05-2.38, *P*=0.038), while *NFκB1*-3297 CT had reduced SMA risk (OR=0.52, 95%CI=0.33-0.81, *P*=0.004). *NFκB1* AT haplotype also had reduced SMA risk (OR=0.61, 95%CI=0.42-0.88, *P*=0.008). Longitudinally, *NFκBIA* -310GA revealed association with increased risk of malaria events (RR=1.13, 95%CI=1.07-1.20, *P*=5.60^{e-05}). The *NFκBIA* AG haplotype increased risk of repeated malaria events both in the binary and the additive status (RR=1.16, 95%CI=1.03-1.31, *P*=0.017 and OR=1.07, 95%CI=1.00-1.13, *P*=0.039, respectively). In contrast, *NFκB1* AC haplotype significantly reduced the risk of malaria episodes (RR=0.93, 95%CI=0.89-0.98, *P*=0.007). Moreover, *NFκBIA* AA haplotype had significant reduction in all-cause mortality (HR=0.37, 95%CI=0.15-0.93, *P*=0.033). Finally, *NFκB1* AT haplotype demonstrated high risk to malaria events over the follow up period (HR=1.08, 95%CI=1.03-1.14, *P*=0.003). These results demonstrate that variations in human *NFκBIA* and *NFκB1* influence pediatric malaria outcomes and all-cause mortality in western Kenya.

955

MECHANISMS BY WHICH GENETIC VARIATION IN ATP2B4 MAY PROTECT FROM SEVERE MALARIA

Fatou Joof[1], Elena Hartmann[2], Alison Jarvis[2], Alhassan Colley[1], Marion Avril[3], Andrew M. Prentice[1], Carla Cerami[1]

[1]*Medical Research Council, The Gambia Unit at London School of Tropical Medicine and Hygiene, Banjul, Gambia,* [2]*The University of Manchester, Manchester, United Kingdom,* [3]*University of Washington, Seattle, WA, United States*

Recent genome wide association studies (GWAS) identified single nucleotide polymorphisms (SNPs) in several human genes associated with decreased risk of severe malaria. Polymorphisms on *ATP2B4* which encodes PMCA4b, the primary regulator of erythrocyte calcium concentration, have one of the strongest and most reproducible associations. These SNPs are in linkage disequilibrium with each other and are common in the Gambian population (MAF=0.3). We used a recall by genotype approach to investigate the mechanism by which polymorphisms on *ATP2B4* could confer malaria protection. For this, we recruited subjects donating red blood cells (RBCs), from our database of pre-genotyped individuals with homozygote wild type (n= 30), heterozygote (n= 35) and homozygote mutant (n= 31) alleles of *ATP2B4.* We used flow cytometry-based methods to assess the protein expression of PMCA4b and calcium flow, as well as parasite growth and invasion in RBCs from individuals. In addition, we used an *in-vitro* model to assess the binding phenotype using parasitized RBCs and examined corresponding *var* gene expression. We observed a reduced rate of PMCA4b expression, calcium channel activity and decreased *Plasmodium falciparum* growth rate in RBCs from individuals with homozygote mutant genotype in comparison to homozygote wild type and heterozygote (P<0.05). However, no difference in invasion, binding or *var* gene expression was observed between parasites grown in RBCs from each of the three genotypes. This data shows that *ATP2B4* polymorphisms impair the expression and function of PMCA4b, which affects malaria infection outcome by decreasing parasite density. Further work on the effect of the polymorphisms on *P. falciparum* multiplication rate and life cycle is currently under investigation. Our results presented here provide the first experimental validation of GWAS reporting protective effects of SNPs in *ATP2B4* on malaria pathogenesis and demonstrate the importance of calcium in parasite intra-erythrocytic growth.

956

ERYTHROCYTES CLEARANCE DURING POST-TREATMENT DELAYED HEMOLYSIS IN SEVERE MALARIA: THE BIOMECHANICAL HYPOTHESIS

Charlotte Chambrion[1], Mallorie Depond[2], Oussama Mouri[3], Sylvestre Biligui[3], Michael Dussiot[4], Eric Kendjo[3], Aurélie Fricot-Monsinjon[2], Aida Taieb[3], Camille Roussel[1], Jerôme Cros[5], Safi Dokmak[5], Ilhame Tantaoui[3], Nicolas Argy[6], Sandrine Houze[6], Renaud Piarroux[3], Jean-Yves Siriez[7], Sébastien Larréché[8], Marc Thellier[3], Pierre Buffet[1], Papa Alioune Ndour[1]

[1]*INSERM U1134 INTS. French National Reference Center for Malaria. Labex GRex, Paris, France,* [2]*INSERM U1134 INTS. Labex GRex, Paris, France,* [3]*French National Reference Center for Malaria, Hôpital Pitié Salpêtrière, Paris, France,* [4]*Université de Paris, Labex GR-EX, Paris, France,* [5]*APHP Beaujon, Paris, France,* [6]*Hôpital Xavier Bichat-Claude Bernard, French National Reference Center for Malaria, Paris, France,* [7]*Hôpital Robert-Debré, Paris, France,* [8]*Département de Biologie Médicale, Hôpital d'Instruction Des Armées Bégin, Paris, France*

Intravenous artesunate is a first-line treatment for severe malaria. Once ring-circulating *P. falciparum* is killed, the dead parasite is expelled from the RBC in the spleen. The resulting pitted RBC then returns to the circulation. Their elimination few days/weeks later contributes to post-arteminsin delayed hemolysis (PADH). PADH is reported in 5-25% of artesunate-treated patients. Changes in pitted RBC, by infection, treatment then pitting, likely induce alterations leading to their clearance. Preliminary data suggest that part of the massive loss of RBC during PADH is by opsonization likely *via* a specific recognition of parasite antigens or lysis by complement. However pitted RBC may be less deformable and mechanical retained by the spleen. Imaging flow cytometry (allowing morphological features analysis) of pitted RBC obtained from severe malaria patients (n=32) or generated through an *ex-vivo* human spleen model (n=2) showed up to 12.75% reduction of surface area, compared to never infected RBC. By ektacytometry, patient's RBC after treatment were 6% (n=6) less deformable than those from healthy donors (n=8) Finally, through a microsphiltration device mimicking spleen filtration, we measured the filterability of pitted RBC and iRBC during the follow up of severe malaria patients subjected to delayed hemolysis compared to control RBC. The result showed that on average, iRBC (n=8) were 6.5 more retained than healthy RBC (n=4) and pitted RBC (n=9) were 12.6 more retained than healthy RBC. These results suggest that morphological alterations of pitted RBC could lead to the reduced RBC deformability and impair their ability to cross the splenic slits. Taken together, this data support that both immunological and mechanical processes likely contribute to the massive elimination of RBC during post artemisinins delayed hemolysis. Deciphering PADH's pathogenesis may allow to prevent and anticipate an appropriate care of the patient, organ failure linked to worsening of the anemia.

957

DUFFY-NEGATIVE AND DUFFY-POSITIVE *PLASMODIUM VIVAX* SHARED SIMILAR GENE POOL INDICATIVE OF FREQUENT TRANSMISSION IN EAST AFRICA

Daniel Kepple[1], Alfred Hubbard[2], Karen Lopez[2], Kareen Pestana[1], Musab Ali[3], Beka Raya[4], Daniel Janies[2], Muzamil Hamid[3], Delenasaw Yewhalaw[4], Eugenia Lo[1]

[1]*Biological Sciences, University of North Carolina at Charlotte, Charlotte, NC, United States,* [2]*Bioinformatics and Genomics, University of North Carolina at Charlotte, Charlotte, NC, United States,* [3]*Department of Parasitology and Medical Entomology, Institute of Endemic Diseases, University of Khartoum, Khartoum, Sudan,* [4]*Tropical Infectious Disease Research Center, Jimma University, Jimma, Ethiopia*

Plasmodium vivax malaria has been a neglected tropical disease in Africa due to lower occurrences and fatalities compared to *P. falciparum*. This is largely due to the parasite requiring the Duffy antigen/chemokine receptor for erythrocyte invasion, which most Africans lack. Recent

reports have shown *P. vivax* cases in East Africa have been increasing at alarming rates and a portion of these cases were detected in Duffy-negative individuals. This study utilized eight microsatellite markers to examine *P. vivax* symptomatic samples collected from six study sites, three in Ethiopia and three in Sudan, to determine the parasite genetic variation and transmission patterns in the Duffy-negative and Duffy-positive populations. Specifically, we tested if the Duffy-negative *P. vivax* from Ethiopia shares similar gene pool with those from Sudan, and if they are genetically similar to the Duffy-positive *P. vivax* from the same region. We found no significant linkage disequilibrium amongst all genetic loci. Both Duffy-negative and Duffy-positive *P. vivax* revealed similar level of polyclonal infections. Duffy-positive *P. vivax* generally showed higher genetic variation, but Duffy-negative *P. vivax* showed higher genotypic evenness. Overall, *P. vivax* in Duffy-negatives was not clearly differentiated from Duffy-positives that coexisted in the same area. Slight differences were observed in the Duffy-positive *P. vivax* between Ethiopia and Sudan, but the genetic composition of Duffy-negative *P. vivax* was fairly similar. A weak isolation-by-distance pattern was detected among all study sites, suggestive of frequent gene flow. The association between gene flow and landscape features was further explored by fitting resistance surfaces based on elevation, road density, and land cover to genetic distances. These findings collectively indicated *P. vivax* can transmit between Duffy-positive and Duffy-negative individuals without geographical or biological barriers. Our study provides critical insight on *P. vivax* epidemiology in Africa and establishes a foundation for future investigations on the transmission mechanisms of Duffy-negative *P. vivax*.

959

CORONAVIRUS SURVEILLANCE IN CONGO BASIN WILDLIFE DETECTS RNA OF MULTIPLE SPECIES CIRCULATING IN BATS AND RODENTS

Christian E. Lange[1], Charles Kumakamba[2], Fabien R. Niama[3], Maria Makuwa[4], Amethyst Gillis[5], Matthew LeBreton[6], David McIver[7], Damien Joly[8], Karen Saylors[4]

[1]*Metabiota, Inc, San Francisco, CA, United States,* [2]*Metabiota, Inc, Kinshasa, Democratic Republic of the Congo,* [3]*National Laboratory of Public Health, Brazzaville, Republic of the Congo,* [4]*Labyrinth Global Health, Inc, St Petersburg, FL, United States,* [5]*Development Alternatives, Inc., Washington, DC, United States,* [6]*Mosaic, Yahounde, Cameroon,* [7]*University of California, San Francisco, CA, United States,* [8]*British Columbia Ministry of Environment and Climate Change Strategy, Victoria, BC, Canada*

Coronaviruses play an important role as pathogens of humans and animals, and the emergence of epidemics like SARS, MERS and COVID-19 is closely linked to zoonotic transmission events from primarily wild animals. Bats have been proposed as a rich source of coronaviruses with the potential to infect humans, with other animals serving as intermediate or alternative hosts or reservoirs. Host diversity may in this context be an important contributor to viral diversity and thus the potential for zoonotic events. So far only very limited research has been done in Africa on this topic, and especially the situation in the Congo Basin where there is frequent contact between humans and wildlife remains understudied. We thus sampled and PCR tested 3561 wild animals for coronavirus RNA, with a focus on bats, rodents, and primates. We found coronavirus RNA in 121 animals, of which all but two were bats. Bats sampled in the local calendric wet season were significantly more likely to be coronavirus RNA positive. The detected sequences correspond to 15 Alpha and 6 Beta coronaviruses, with some of them being very similar to known coronaviruses and others being more unique and potentially representing novel viruses. In seven of the bats, RNA resembling viruses related to human types 229E or NL63 was detected. The findings reiterate the potential of coronavirus spillover, especially in regions with a high diversity of bats and close human contact with them and reinforces the need for consistent surveillance following COVID-19.

960

CLINICAL AND EPIDEMIOLOGIC FEATURES ASSOCIATED WITH MILD OR EARLY COVID-19 IN AN OUTPATIENT SETTING

Jessica K. Fairley[1], Taylor Landay[2], Amy Sherman[1], Henry M. Wu[1], Matthew H. Collins[1]

[1]*Emory University School of Medicine, Atlanta, GA, United States,* [2]*Emory Rollins School of Public Health, Atlanta, GA, United States*

As the SARS-CoV2 virus spreads across the world and we brace for a long battle, it is crucial to recognize signs and symptoms of mild or early infection in order to triage, test, and interrupt transmission of infection. Current data are limited to retrospective datasets of hospitalized patients. We present data from the first 78 subjects prospectively enrolled in a study analyzing clinical and epidemiologic factors of outpatients presenting with respiratory symptoms during the height of the COVID-19 pandemic. Symptomatic patients were recruited from an outpatient testing site. After giving verbal consent, they were enrolled and administered a questionnaire to collect data on demographics, potential exposures, medical history and symptoms. A nasal pharyngeal swab was taken and tested for SARS-CoV2 by PCR. Descriptive and bivariate analyses were conducted to compare those who tested positive and those who tested negative. Fifteen (19.2%) tested positive for SARS-CoV2, 62 (79.5%) tested negative, and 1 (1.3%) indeterminate. Two-thirds were female, 76% were healthcare workers, and more than half tested (52%) had contact with a confirmed case. Fever was more frequent among cases (62.5%) than non-cases (25.8%), p-value = 0.001. While based on only 30 patients with full survey data, other notable symptoms in cases vs non-cases were cough (80% vs 60%, p = 0.40), nasal congestion (100% vs 44%.p = 0.02), runny nose (0% vs 44%, p = 0.06), loss of smell (43% vs 0%, p < 0.001), and loss of taste (43% vs 0%, p < 0.001). This study shows a high positivity rate of tested patients in this screening clinic at the height of the COVID-19 outbreak in our metropolitan area. Further recruitment and data acquisition are ongoing; however, initial results suggest certain clinical features at presentation such as loss of taste and / or smell are predictive of a COVID-19 positive test. Presence of runny nose tended to favor a diagnosis other than COVID-19. As the pandemic starts to abate, results from this study will assist clinicians and public health officials to better triage testing and isolation countermeasures to prevent recurrent peaks of infection.

961

COMPREHENSIVE IMMUNE PROFILING OF CHIKUNGUNYA AND DENGUE VIRAL RESPONSES USING A NOVEL MINIATURIZED AUTOMATED WHOLE BLOOD CELLULAR ANALYSIS SYSTEM AND MASS CYTOMETRY (CYTOF) IN A PEDIATRIC COHORT IN MSAMBWENI, KENYA

Sangeeta Kowli[1], Amy Krystosik[2], Matthew Hale[3], Francis Mutuku[4], Jael Sagina[5], Saidi Lipi[5], Phillip K. Chebii[5], Priscillah W. Maina[5], Elysse Grossi-Soyster[2], Holden T. Maecker[1], **Desiree LaBeaud**[2]

[1]*Human Immune Monitoring Center, Stanford University, School of Medicine, Stanford, CA, United States,* [2]*LaBeaud Laboratory, Stanford University, School of Medicine, Department of Pediatrics, Division of Infectious Disease, Stanford, CA, United States,* [3]*4Smart Tube Inc., Menlo Park, CA, United States,* [4]*Department of Environment and Health Sciences, Technical University of Mombasa, Mombasa, Kenya,* [5]*Vector-borne disease control unit, Msambweni field station, Kwale County, Kenya*

Chikungunya (CHIKV) and dengue (DENV) are mosquito-borne viruses that cause debilitating global epidemics often in remote developing nations without ample capacity to fully interrogate the circulating viral immune response. To fill this gap, we created a novel miniaturized automated Smart Tube system that incorporates stimulation, lysis and fixation of 250 µl of whole blood, which can then be stored and shipped for further high throughput analysis such as CyTOF. In this study, we field tested our novel system using an ongoing pediatric cohort (median age, 8 years) in Msambweni, Kenya, known to be previously exposed to CHIKV and/or DENV by prior PCR and/or ELISA testing. In March 2018, 133 whole blood samples were processed in Kenya using the novel Smart Tube

system for three conditions: no stimulation and stimulation with CHIKV and DENV peptide pools. Frozen samples were shipped to Stanford for further immune analysis by CyTOF. A panel of ~40 markers was used to stain the fixed whole blood samples to identify virus-specific memory T cell responses as well as general immune phenotypes including activation marker expression. We have tested 81 participant samples using CyTOF. We performed 2way-ANOVA and our analysis reveals significant cytokine response of IFNγ ($p<0.01$ and $p<0.0001$; 57% ; 67% participants) and TNFα ($p<0.0001$; 60%; 63% participants) by the γδ T cells to CHIKV and DENV respectively. We also observe a significant TNFα response in the CD8+ TEMRA memory subset to DENV, albeit to a lesser degree compared to γδ T cells. No significant CD4+ or CD8+ IFNγ, TNFα and IL-2 responses were observed. To confirm our CyTOF findings, we will next, perform flow cytometry on the remaining study samples, using a targeted panel. Our findings show that our novel automated SMART TUBE system can permit detailed immune function evaluation, especially in pediatric populations where sample volumes are limiting as well as in remote settings. The application of this system can be extended to other viral and infectious disease studies, to better understand cellular immune responses in disease in pediatric populations that can aid in vaccine trial monitoring.

962

RISK FACTORS ASSOCIATED WITH CHIKUNGUNYA AND DENGUE EXPOSURE AMONG CHILDREN IN COASTAL AND WESTERN KENYA

Shama Cash-Goldwasser[1], Jonathan Altamirano[1], Bryson Ndenga[2], Loice Lwamba[2], Sandra Musaki[2], Charles Muiruri Ng'ang'a[3], Said Lipi Malumbo[3], Jael Sagina Amugongo[3], Francis Mutuku[4], A. Desiree LaBeaud[1]

[1]*Stanford University, Stanford, CA, United States,* [2]*Kenya Medical Research Institute, Kisumu, Kenya,* [3]*Vector Borne Disease Control Unit, Msambweni, Kenya,* [4]*Technical University of Mombasa, Mombasa, Kenya*

Chikungunya virus (CHIKV) and dengue virus (DENV) are important reemerging diseases in sub-Saharan Africa but our poor understanding of factors that drive transmission hampers the development of prevention programs. To address this knowledge gap, we conducted a cohort study on the burden and transmission of CHIKV and DENV among children Kenya. From 2014 through 2018, we followed healthy children between 1 – 19 years of age in 4 communities that comprise urban and rural areas in both coastal and western Kenya. Upon enrollment and at 6-month follow up visits, we collected blood samples and administered risk factor questionnaires. Blood was tested by IgG ELISA for antibodies to CHIKV and DENV. Over the five years of the study, 3,905 children completed 14,190 visits from which CHIKV and/or DENV serologic results were obtained. Of 13,210 CHIKV test results obtained, 777(5.9%) were positive, and of 14,098 DENV test results obtained, 430(3.1%) were positive. Children that were CHIKV seropositive were older than those who were not (9.7 years versus 8.5 years); a similar trend was observed for DENV (10.8 years versus 8.5 years). There was more CHIKV seropositivity in the west (10.3%) than on the coast (2.0%) ($P < 0.001$) and in rural (10.1%) than in urban (2.4%) areas ($P < 0.001$). There was more DENV seropositivity on the coast (4.4%) than in the west (1.9%) ($P < 0.001$) and in rural (4.7%) than in urban areas (1.7%) ($P < 0.001$). Of 2,547 children with at least two CHIKV results, 199 (7.8%) seroconverted; of 2,755 children with at least two DENV results, 126 (4.5%) seroconverted. CHIKV seroconversion was more common in the west (7.3%) than on the coast (3.8%) and in rural (9.3%) versus urban (2.1%) settings. DENV seroconversion was associated with rural (4.9%) versus urban (2.0%) residence. Time-series analysis of factors associated with seroconversion is ongoing. These results show that rural residence is associated with risk for both viruses; in other settings, urban residence has been associated with CHIKV/DENV, thought to be due to the habitat preferences of the primary mosquito vector. Our findings have important implications for disease prevention efforts.

964

A MONOCLONAL ANTIBODY MAPPING TO THE FUSION LOOP OF EASTERN EQUINE ENCEPHALITIS VIRUS E1 GLYCOPROTEIN CROSS-NEUTRALIZES VENEZUELAN EQUINE ENCEPHALITIS VIRUS *IN VITRO* BY SEVERAL MECHANISMS

Amanda E. Calvert[1], Susan L. Bennett[2], Rachel H. Fong[3], Benjamin J. Doranz[3], John T. Roehrig[2], Carol D. Blair[2]

[1]*Centers for Disease Control and Prevention, Fort Collins, CO, United States,* [2]*Colorado State University, Fort Collins, CO, United States,* [3]*Integral Molecular, Philadelphia, PA, United States*

The encephalitic alphaviruses, eastern equine encephalitis virus (EEEV), western equine encephalitis virus (WEEV) and Venezuelan equine encephalitis virus (VEEV) cause sporadic outbreaks in the U.S., Central and South America. These viruses are a biodefense concern because of their low human infective dose and infectivity by aerosol, with no prophylactic or therapeutic treatment available. Monoclonal antibodies (MAbs) with the ability to strongly neutralize these viruses are virus type-specific, inhibiting virus proliferation by blocking attachment and internalization controlled by the E2 glycoprotein. MAbs that recognize the E1 glycoprotein are known to be weakly neutralizing but have been shown to protect from disease in animal models. Here, we investigate the *in vitro* mechanism of neutralization of EEEV murine MAb 1A4B-6, a broadly alphavirus cross-reactive MAb able to neutralize VEEV *in vitro*. 1A4B-6 was able to inhibit virus growth in cell culture at a pre-attachment step but was unable to inhibit virus entry at a post-attachment step. This MAb was also able to inhibit virus egress from infected cells, consistent with quantitative flow cytometry data showing the abundance of this epitope on the surface of infected cells. Alanine-scanning mutagenesis identified C96 and N100 in the fusion loop of E1 as critical residues for binding. The potential for broadly cross-reactive MAbs with limited virus neutralizing activity *in vitro* merits further exploration for use as anti-viral prophylaxis or therapy.

965

DETERMINANTS OF ARBOVIRUS VECTOR DENSITY AS A MEASURE OF TRANSMISSION RISK IN REGIONS OF RECENT ZIKA VIRUS INTRODUCTION IN THE AMERICAS

Benoit Talbot[1], Beate Sander[2], Camila González[3], Varsovia Cevallos[4], Marcos Miretti[5], Mauricio Espinel[6], Jianhong Wu[7], María Cristina Carrasquilla Ferro[3], Mario Iván Ortiz Yanine[3], Denisse Benítez[4], Patricio Ponce[4], Neris Gauto[8], Karen López[8], Claudio Carissimo[8], Fabián Zelaya[8], Sergio Litwiñiuk[5], Manisha A. Kulkarni[1]

[1]*University of Ottawa, Ottawa, ON, Canada,* [2]*University Health Network, Toronto, ON, Canada,* [3]*Universidad de los Andes, Bogota, Colombia,* [4]*Instituto Nacional de Investigación en Salud Pública, Quito, Ecuador,* [5]*UNaM-CONICET, Posadas, Argentina,* [6]*Universidad Laica Elroy Alfaro de Manabí, Manta, Ecuador,* [7]*York University, Toronto, ON, Canada,* [8]*Municipalidad de Posadas, Posadas, Argentina*

Globalization has increased the exposure of world populations to several vector-borne and zoonotic diseases, including tropical arboviruses. The global impact of Zika virus in Latin America and the Caribbean has drawn renewed attention to other circulating mosquito-borne viruses in this region, such as dengue and chikungunya viruses. Our objective was to assess socioecological factors associated with *Aedes* vector density as a measure of arboviral transmission risk in three regions of recent Zika virus introduction: Posadas, Argentina; Ibagué, Colombia; and Manta, Ecuador, in order to inform disease mitigation strategies. We monitored *Aedes* mosquito populations over 12 months starting in 2018 in a total of 1152 randomly selected households across four neighborhoods per site using kairomone traps, light traps, resting traps, and aspirators, and tested pooled specimens for Zika, dengue and chikungunya viruses using standardized qPCR assays. For each sampled household, we collected socio-economic and microenvironmental data using structured questionnaires. We used generalized mixed-effects Poisson regression analyses to identify predictors of *Aedes* density, with month, site and neighborhood as random-effects variables. Mean female *Aedes* mosquito

density per household was 2.10. No pools tested positive for Zika, while 5-14 pools were positive for dengue and/or chikungunya in each study site. The lowest sextile of household wealth (Incidence rate ratio, IRR = 1.81), the number of household occupants (IRR = 1.08) and presence of holes in the household structure used by mosquitoes as points of entry (IRR = 1.25) were associated with a higher number of female *Aedes* mosquitoes per household. Knowledge of how arboviruses are transmitted (IRR = 0.85) and regular emptying of water containers by occupants (IRR = 0.83) were associated with lower female *Aedes* mosquito density. Our study addresses the complexities of arboviruses of global significance at the interface between humans, vectors and the environment, and identifies parameters that can be used to inform future mitigation strategies.

966

CARING FOR CHILDREN EXPOSED TO ZIKA VIRUS PRENATALLY

Viviana Rosario-Villafañe, Irelis C. Repollet-Carrer, Marilyn Borges-Rodríguez, Paola B. Velázquez-Gonzàlez, Vanessa Rivera-Amill, Mary Rodriguez-Rabassa, **Luisa I. Alvarado-Domenech**

Ponce Health Sciences University, Ponce, PR, United States

Children exposed to Zika virus (ZIKV) prenatally are at risk for adverse developmental outcomes. Caring for children with developmental delay can cause parental distress with a potential impact on the mental health of caregivers. The Pediatric Outcomes of Prenatal Zika Exposure cohort study provides developmental and behavioral follow-up assessments to 59 children exposed to ZIKV prenatally in Puerto Rico. This cross-sectional analysis aims to describe maternal demographics and psychological distress. Thirty-five mothers of the study cohort completed self-reported surveys exploring depression (Beck Depression Inventory-BDI), anxiety (Beck Anxiety Inventory-BAI), and resilience (Maternal Resilience Scale). Descriptive analyses revealed: 27 (77%) are married or living together with the child's father, and 28 (80%) serve as primary caregivers for their children aged 24- or 36- months old. Nearly half (16, 46%) have an educational attainment of high school or technical certificate. Also, the majority receive government aid (32, 91%), have public health insurance (26, 74%), are unemployed (18, 51%), and have an income below the poverty level (28, 80%). Six (17%) mothers had moderate or severe levels of depression (BDI), eight (23%) had moderate or severe levels of anxiety (BAI), and nine (26%) had high scores in the resilience scale. However, 13 (37%) mothers reported feelings of distress when they realize each day it becomes more difficult to raise their child. Depression and anxiety scores were significantly negatively correlated with resilience scores (r= -0.45, $p<0.01$ and r= -0.42, $p<0.01$, respectively). Psychosocial vulnerabilities were identified in mothers caring for their children with ZIKV prenatal exposure that can affect their mental health. Such vulnerabilities might serve as risk factors for developmental delay in children due to difficulties in the ability to assist their interaction with the environment and to provide learning opportunities. Early detection, prevention, and intervention on emotional distress faced by these mothers might facilitate nourishing maternal-infant interaction and parenting skills.

967

RAPID HOST ADAPTATION AND EMERGENCE OF A VIRULENCE-ENHANCING MUTATION DURING SERIAL VERTEBRATE TRANSMISSION OF ZIKA VIRUS

Kasen Riemersma[1], Anna Jaeger[2], Chelsea Crooks[1], Katarina Braun[1], James Weger-Lucarelli[3], Greg Ebel[4], Thomas Friedrich[1], Matthew Aliota[2]

[1]*University of Wisconsin, Madison, WI, United States,* [2]*University of Minnesota, St. Paul, MN, United States,* [3]*Virginia Tech, Blacksburg, VA, United States,* [4]*Colorado State University, Fort Collins, CO, United States*

Alternating mosquito-vertebrate transmission broadly restricts arbovirus evolution and adaptation under laboratory conditions. Zika virus (ZIKV) has the unusual ability to bypass the mosquito vector and directly transmit human-to-human via sexual and vertical routes. Direct transmission of ZIKV has also been reported via blood transfusion. The impact of direct transmission on ZIKV evolution and adaptation to vertebrate hosts or mosquito vectors is unknown. To address this, we modeled direct transmission chains by serially passaging molecularly barcoded ZIKV in mice and *Aedes aegypti* mosquitoes via needle inoculation. We also alternately passaged barcoded ZIKV between mice and mosquitoes via natural blood-feeding transmission. Phenotypic changes were measured in mice and mosquitoes to assess host and vector adaptation. Deep sequencing of virus barcodes and whole ZIKV genomes was used to track changes in ZIKV population structure and composition, and to characterize the selective processes at play. These in-depth phenotypic and genetic analyses revealed rapid host adaptation of ZIKV during serial passage in mice coincident with the emergence of an amino acid substitution, NS2A A117V, previously shown to confer enhanced virulence in mice. In contrast, ZIKV demonstrated little host adaptation following serial passage in mosquitoes or alternating passage, and NS2A A117V was not observed during any mosquito or alternating passage. In general, ZIKV evolution in mice was more convergent and subjected to more relaxed purifying selection than in mosquitoes or alternate passages. Although ZIKV evolution during alternating passage was predictably limited, its trajectory was divergent between replicates, suggesting that genetic drift is the predominant selective process during alternating passage. These findings demonstrate that, under laboratory conditions, direct host-to-host transmission enables ZIKV to rapidly host-adapt and increase virulence. As a result, prevention of direct human transmission chains via sexual routes or blood transfusion may be paramount to inhibit human adaptation of ZIKV.

968

EARLY FETAL DEMISE FOLLOWING INTRAVAGINAL ZIKA VIRUS CHALLENGE IN RHESUS MACAQUES

Christina M. Newman[1], Alice F. Tarantal[2], Christopher J. Miller[2], David H. O'Connor[1]

[1]*University of Wisconsin-Madison, Madison, WI, United States,* [2]*California National Primate Research Center, Davis, CA, United States*

Zika virus (ZIKV) circulated in parts of Africa and Southeast Asia for decades but remained understudied due to its lack of association with significant human disease. It was not until 2015, when ZIKV emerged in Brazil and rapidly spread throughout the Americas, that ZIKV became associated with Guillain-Barré syndrome and congenital ZIKV syndrome (CZS), a constellation of severe pregnancy outcomes including congenital microcephaly and fetal demise. ZIKV is an arthropod-borne virus (arbovirus) and is primarily transmitted by *Aedes* species mosquitoes; however, ZIKV can also be sexually transmitted. During the initial epidemic and in places where ZIKV is now considered endemic, it is difficult to disentangle the risks and contributions of sexual versus vector-borne transmission to adverse pregnancy outcomes. We hypothesized that sexual transmission of ZIKV contributes to adverse pregnancy outcomes and could pose an underrecognized threat to fetal and maternal health. We challenged three rhesus macaques (*Macaca mulatta*) three times intravaginally with 1×10^7 PFU of a low passage, African lineage ZIKV strain in the early first trimester (30 days gestation). Samples were collected from all animals initially on days three through ten post challenge, followed by twice, and then once weekly sample collection; ultrasound examinations were performed weekly. All three dams had ZIKV RNA detectable in plasma by day five post challenge. At approximately 50 days gestation (21 days post challenge), two of the three dams were found with nonviable fetuses by ultrasound. These results suggest that sexual transmission of ZIKV may increase the risk of early fetal demise.

969

THE INFLUENCE OF TYPE 1 INTERFERON ON PROGRAMMING THE ZIKA VIRUS SPECIFIC T-FOLLICULAR HELPER CELL AND B CELL RESPONSE

Tara L. Steffen, Mariah Hassert, Amelia K. Pinto, James D. Brien

Saint Louis University, Saint Louis, MO, United States

Zika virus (ZIKV) is a significant public health concern with its dual mode of transmission and association with explosive outbreaks, thus necessitating the development of a vaccine that will drive a protective antibody response. Type I interferon (IFN) is an essential cytokine in establishing the host antiviral state and informing the adaptive immune response by upregulating components of antigen presentation and other cytokines; however, it also has potent anti-proliferative effects. We hypothesize that type 1 IFN acts as a negative regulator of the antigen specific T-follicular helper cell (TFH), B cell, and polyclonal antibody response by limiting proliferation. Using a type I IFN receptor deficient mouse model ($IFNAR1^{-/-}$) we show that disrupting the type 1 IFN signaling pathway at the receptor increases the frequency of TFHs, germinal center B cells, and plasmablasts after ZIKV infection. This increase in frequency functionally impacts the polyclonal antibody response, resulting in an increase in the neutralization capacity. To determine if deficiency in type 1 IFN signaling is advantageous to the antigen specific B cell response, we evaluated responses to infection and vaccination with replicative ZIKV and dengue serotype-2 or non-replicative vaccine constructs. Furthermore, we investigate a potential link between type 1 IFN signaling deficiency in dendritic cells with altered humoral immune responses to viral infection and vaccination. Using antigen specific methods, we evaluated differences between neutralization and enhancement capacity of the polyclonal response *in vitro*, variances in antibody binding characteristics to structural proteins, and induction of antigen specific TFH cells and B cells in C57BL/6 mice, $IFNAR1^{-/-}$ mice, and dendritic cell specific knockouts (CD11c cre $IFNAR^{fl/fl}$) after both infection and vaccination. This study provides crucial information regarding the role of type I IFN in regulating the virus specific B cell response and the possible impact of type I IFN during vaccine induced antibody responses.

970

A ROBUST NONHUMAN PRIMATE PREGNANCY MODEL TO TEST ZIKA VIRUS COUNTERMEASURES

Dawn M. Dudley[1], Keisuke Yamamoto[1], Phoenix M. Shepherd[1], Meghan E. Breitbach[1], Christina M. Newman[1], Kathryn Bach[1], Mason I. Bliss[2], Sierra L. Rybarczyk[2], Emily Sneed[2], Heather A. Simmons[2], Andres Mejia[2], Michael K. Fritsch[1], Emma L. Mohr[1], Karla K. Ausderau[1], Matthew T. Aliota[3], Thomas C. Friedrich[1], David H. O'Connor[1]

[1]*University of Wisconsin-Madison, Madison, WI, United States,* [2]*Wisconsin National Primate Research Center, University of Wisconsin-Madison, Madison, WI, United States,* [3]*University of Minnesota, St. Paul, MN, United States*

Congenital Zika syndrome (CZS) is the most important adverse outcome associated with Zika virus (ZIKV) infection. Despite ongoing outbreaks in many countries, there are no FDA approved ZIKV treatments or vaccines to mitigate CZS. Part of the difficulty in developing these important tools is the lack of a pregnant nonhuman primate model that consistently leads to CZS. The natural ZIKV transmission model in nonhuman primates (NHPs), using a physiologically relevant dose of ZIKV-Puerto Rico (PR) (strain PRVABC59), results in adverse fetal outcomes in approximately one in five pregnancies. While this rate of adverse outcomes mimics those seen in humans, it is difficult to enroll enough NHPs to study efficacy in a model with infrequent outcomes. More frequent adverse pregnancy events have been observed in mice infected with an African strain of Zika virus-DAK AR41524 (ZIKV-Dak)- and in nonhuman primate models using extremely high doses of Asian-lineage ZIKV. We hypothesized that infecting pregnant NHPs with a high dose of ZIKV-Dak would result in more frequent adverse fetal outcomes. We therefore infected 5 pregnant rhesus macaques with $1x10^8$ plaque forming units (PFUs) of ZIKV-Dak on gestational day (GD) 45. High dose infection with ZIKV-Dak led to earlier peaks in plasma viremia, but not significantly higher peak viral loads nor longer durations of maternal viremia compared to animals infected with $1x10^4$ PFUs of ZIKV-Dak or ZIKV-PR. Two of five pregnancies ended in fetal demise at 17 and 21 days post infection, whereas 4/4 infants survived from dams infected with $1x10^4$ PFU of ZIKV-Dak. ZIKV RNA was detected in all tissues collected from pregnancies that ended in demise (fetuses and maternal fetal interface). Two of five pregnancies went to full term and these ZIKV-Dak-exposed infants had lower trending orientation scores compared to infants exposed to ZIKV-PR or mock controls. One pregnancy is currently ongoing. These data suggest that inoculation with a high dose of ZIKV-Dak increases the incidence of severe adverse fetal outcomes and improves the feasibility of testing ZIKV countermeasures in a translational animal model.

971

CLARIFYING THE CONGENITAL ZIKA SYNDROME PHENOTYPE AND EXPANDING TO CONGENITAL ZIKA SPECTRUM

Laura D. Zambrano[1], Augustina Delaney[1], Charles E. Rose[1], Suzanne Gilboa[1], Van Tong[1], Miguel Valencia[2], Nicole M. Roth[1], Janet Cragan[1], Jazmyn Moore[1], J. Erin Staples[1], Margaret Honein[1], Cynthia Moore[1]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Puerto Rico Department of Health, San Juan, PR, United States*

Congenital Zika syndrome (CZS) is a term used to describe the pattern of anomalies in infants due to congenital Zika virus (ZIKV) infection. To date, published reports of infants with these anomalies have been primarily small case series of the most severely affected infants and attempts to determine the CZS phenotype have been based on those reports. Lack of a standard definition has led to inconsistencies in the term's use in the literature and uncertainty about the full spectrum of anomalies, limiting the application for diagnostic and surveillance purposes. We first sought to understand which defects co-occur with possible congenital ZIKV infection using data from 415 mother-infant dyads with laboratory evidence of confirmed or presumptive Zika virus infection from the U.S. Zika Pregnancy and Infant Registry, and a comparison group of 4534 mother-infant dyads with no documented or plausible ZIKV infection from the Zika Birth Defects Surveillance System. Using k-means cluster analysis and subsequent canonical discriminant analysis, a clinically distinct phenotype emerged as a single cluster in infants for whom both brain and eye defects were recorded that corresponded to evidence of confirmed or presumptive ZIKV infection. A combination of six defects (sub-cortical calcifications, chorioretinal atrophy/pigmentary anomalies, arthrogryposis or clubfoot, cerebral atrophy or ventriculomegaly, abnormal cortical gyration, and optic nerve atrophy/pallor/other optic nerve abnormalities) predicted the presence of laboratory evidence (area under the receiver operating characteristics curve: 0.95, 95% confidence interval: 0.90-0.99). Further analyses to develop a scoring system to weigh the evidence of specific congenital anomalies, separately and in combination, that are consistent with laboratory evidence of congenital ZIKV infection are in progress. A quantitatively determined spectrum of Zika-associated anomalies will enable clinicians to provide patient-level probabilities of congenital ZIKV infection, based on the presence of specific combinations of congenital anomalies in the absence of laboratory information.

972

CROSS BORDER SURVEILLANCE IN SOUTHERN ANGOLA: AN ANALYSIS OF RESULTS ACHIEVED AFTER THREE YEARS (2017-2020) OF ACTIVITY IMPLEMENTATION

José Franco Martins[1], Joana Rosário[2], Rukaaka Mugizi[2], Paulo Máquina[2], Nyasha Mwendera[3], Anna Johansson[4], Sergio Lopes[5], Julio Ramirez[5]

[1]*National Malaria Control Program, National Directorate for Public Health, Ministry of Health Angola, Luanda, Angola,* [2]*Elimination 8 Secretariat,*

Luanda, Angola, [3]Elimination 8 Secretariat, Windhoek, Namibia, [4]ADPP Angola – Ajuda de Desenvolvimento de Povo para Povo, Luanda, Angola, [5]The MENTOR Initiative, Luanda, Angola

Malaria is one of the main public health problems in Angola. Nomad and migrant populations in southern border with Namibia are also affected by this disease. In 2017, under the leadership of the Angolan National Malaria Control Program (NMCP), SADC Elimination 8 (E8) and partners started a project in two provinces of southern Angola aiming to contribute to the reduction of cross border malaria between Angola and Namibia. Several strategies were implemented including the set up of 8 border posts to diagnose, treat and monitor malaria in nomad and migrant populations along the border. This abstract aims to present the results of these actions throughout time. A retrospective descriptive study was conducted looking at data collected in these border posts between September 2017 and March 2020. Temporal trends on positive ratios were checked and other key variables were looked at (gender, age, residence). We looked at potential explanatory variables for the decrease verified in positivity ratios throughout time in all border posts. More than 300.000 malaria tests were conducted during the period of analysis (30 months). Of these, 8% were positive. At the beginning of the intervention the overall positivity ratio was 18.4% but extremely heterogeneous between the two provinces (above 40% in Cuando Cubango and lower than 5% in Cunene). By the end of March, the overall positivity was around 1.4% (1.8% in Cuando Cubango and 1.4% in Cunene). An overall reduction of 92.4% in positivity was verified since the beginning of the project. Strategies used to control and eliminate malaria in this area have been effective and produced some interesting results. There is a clear need to reorient current strategies towards active surveillance for malaria elimination and ally these to effective vector control and case management to guarantee sustained gains towards malaria elimination in southern Angola.

973

A MALARIA MICRO-STRATIFICATION APPROACH TO SUPPORT EVIDENCE-BASED DECENTRALIZED MALARIA CONTROL PLANNING AND IMPLEMENTATION IN MAINLAND TANZANIA

Sumaiyya G. Thawer[1], Victor Alegana[2], Frank Chacky[3], Sigsbert Mkude[4], Peter M. Macharia[5], Samwel Lazaro[6], Ally Mohamed[6], Christian Lengeler[1], Amanda Ross[1], Robert W. Snow[7], Fabrizio Molteni[8], Emilie Pothin[9]

[1]*Swiss Tropical and Public Health Institute/ University of Basel, Basel, Switzerland,* [2]*Population Health Unit, KEMRI-Welcome Trust Research Programme, Nairobi, Kenya,* [3]*National Malaria Control Programme,Ministry of Health, Community Development, Gender, Elderly, and Children, Dodoma, United Republic of Tanzania,* [4]*Swiss Tropical and Public Health Institute, Dar es Salaam, United Republic of Tanzania,* [5]*5 Population Health Unit, KEMRI-Welcome Trust Research Programme, Nairobi, Kenya,* [6]*National Malaria Control Programme, Ministry of Health, Community Development, Gender, Elderly, and Children, Dodoma, United Republic of Tanzania,* [7]*Population Health Unit, KEMRI-Welcome Trust Research Programme/ Centre for Tropical Medicine and Global Health, Nuffield Department of Clinical Medicine, University of Oxford, Nairobi, Kenya,* [8]*Swiss Tropical and Public Health Institute/ National Malaria Control Programme, Dar es Salaam, United Republic of Tanzania,* [9]*Swiss Tropical and Public Health Institute/ University of Basel/Clinton Health Access Initiative, Basel, Switzerland*

Control efforts in mainland Tanzania have led to a decline in the prevalence of malaria infection (*Pf*PR_{6-59mo}) from 18.1% (DHS/MIS 2008) to 7.3% (MIS 2017). As transmission decreases, sub-national stratification becomes crucial for targeting interventions cost-effectively. In line with the World Health Organization (WHO) High Burden to High Impact (HBHI) initiative that calls for the use of quality data for a targeted malaria control approach, mainland Tanzania has employed a country-led data-driven approach to develop a national malaria risk stratification. This approach was originally developed during the revision of the National Strategic Plan 2015-2020. The updated strategies addressed the need for different packages of interventions at operational units of programme delivery (councils) across the spectrum of malaria risk strata. As the country moves towards implementation of these targeted packages, a more granular micro-stratification of malaria risk will become increasingly valuable for targeting community-based interventions. The country's routine health management information system provides data that can be used to define the risk strata of each ward within a council. Estimates of fever test positivity rates, annual parasite incidence rates, and malaria positivity in pregnant women were collated at ward level for the period 2017-2019. A small area estimation method was employed to estimate the malaria risk per indicator per ward after adjusting for seasonality. A framework for classifying these routine indicators into appropriate thresholds was developed and allowed the allocation of 3,312 wards to one of four risk groups: very low, low, moderate and high. The resulting micro-stratification will inform council health managers about the malaria situation in their respective wards, hence supporting an evidence-based decentralised malaria control planning and implementation in the country. Such a framework will provide improvements in the allocation efficiency of malaria funding to maximise future impacts on disease burden.

974

PLASMODIUM MALARIAE INFECTION IS ASSOCIATED WITH ANEMIA AMONG FEBRILE PATIENTS PRESENTING TO AN URBAN EMERGENCY DEPARTMENT IN DOUALA, CAMEROON

Daniel Z. Hodson[1], Yannick M. Mbarga[2], Tatiana Nganso[3], Glwadys Cheteug N[4], Abigail D. Pershing[5], Narcisse M. Nghokeng[2], Martina Wade[6], Carole E. Eboumbou Moukoko[3], Sunil Parikh[6], Yap Boum II[7]

[1]*Yale University School of Medicine, New Haven, CT, United States,* [2]*Douala Military Hospital, Douala, Cameroon,* [3]*Pasteur Center of Cameroun, Yaoundé, Cameroon,* [4]*University of Buea, Buea, Cameroon,* [5]*Yale Law School, New Haven, CT, United States,* [6]*Yale School of Public Health, New Haven, CT, United States,* [7]*Médecins Sans Frontières/ Epicentre, Yaoundé, Cameroon*

Plasmodium malariae (Pm), historically associated with a more benign clinical picture compared to *P. falciparum* (Pf), has also been implicated in off-season fever episodes, chronic infections, albuminuria, and anemia. While recent molecular-based diagnostics across sub-Saharan Africa have uncovered a significant prevalence of Pm, detection often focuses on asymptomatic community samples, leaving numerous gaps in our understanding of the clinical significance of Pm. Between June and November 2018, we recruited 554 febrile patients ≥6 months old presenting the Emergency Department of the district-level Military Hospital in Douala, Cameroon. We assessed for clinical anemia, performed a point-of-care hemoglobin (Hb) measurement, and obtained RBC pellet samples from venous blood. Molecular-based PCR for Pf and Pm was conducted at the Pasteur Center of Cameroon. The WHO uses Hb-based cutoffs for mild, moderate, and severe anemia for each of five categories: children 6 to 59 months, children 5-11 years (yrs), children 12-14 yrs, adult women ≥15 yrs, and adult men ≥15 yrs. Anemia was common according to WHO classification: 17% had mild, 17% had moderate, and 6% had severe anemia. Among the subset of 206 Pf mono-infections, 31 (15%) had mild, 37 (18%) had moderate, and 13 (6%) had severe anemia. Of those with severe anemia, 6 were children 5-11 yrs and 4 were adult women. Among the 15 Pm mono-infections, 4 (27%) had mild and 4 (27%) had moderate anemia. Among the 9 Pf/Pm co-infections, 2 (22%) had mild, 3 (33%) had for moderate, and 1 (11%) had severe anemia. The proportion of participants with anemia of any severity amongst those with Pm (mono or Pf/Pm co-infection) was significantly higher compared to uninfected participants (p=.026). The same comparison for Pf infection was not significant. While direct causality between *P. malariae* infection and anemia cannot be proven, anemia was commonly seen in the setting of Pm mono/mixed infections in individuals presenting to an urban hospital emergency setting. Future research should further elucidate the association of Pm infections with anemia, as well as additional clinical complications.

975

PREDICTORS AND THE EFFECTS ON BIRTH OUTCOMES OF *PLASMODIUM FALCIPARUM* INFECTION IN EARLY PREGNANCY AMONG NULLIPAROUS WOMEN FROM THE DEMOCRATIC REPUBLIC OF THE CONGO, KENYA, AND ZAMBIA

Sequoia Iris Leuba[1], Melissa Bauserman[1], Carl Bose[1], Daniel Westreich[1], Kimberly Powers[1], Andrew Olshan[1], Antoinette Tshefu[2], Waldemar Carlo[3], Elwyn Chomba[4], Edward Liechty[5], Fabian Esamai[6], Saleem Jessani[7], Janet Moore[8], Jennifer Hemingway-Foday[8], Steven Meshnick[1]

[1]*University of North Carolina at Chapel Hill, Chapel Hill, NC, United States,* [2]*Kinshasa School of Public Health, Kinshasa, Democratic Republic of the Congo,* [3]*University of Alabama at Birmingham, Birmingham, AL, United States,* [4]*University Teaching Hospital, Lusaka, Zambia,* [5]*School of Medicine, Indiana University, Indianapolis, IN, United States,* [6]*Department of Child Health and Paediatrics, Moi University School of Medicine, Eldoret, Kenya,* [7]*Aga Khan University, Karachi, Pakistan,* [8]*RTI International, Research Triangle Park, NC, United States*

Each year, in sub-Saharan Africa, 11 million pregnancies are exposed to malaria, and this infection can cause maternal anemia, preterm birth, stillbirth, and low birth weight. However, protective measures including distribution of insecticide-treated nets and intermittent preventive therapy are rarely initiated until the second trimester, leaving pregnant women unprotected from malaria infection in early pregnancy. Few studies have examined malaria infection in early pregnancy. This is the first large multi-site study of malaria in early pregnancy. This is an ancillary study, added to the NICHD Global Network's trial of low-dose aspirin in early pregnancy (ASPIRIN). At early pregnancy (<19 weeks), dried blood spots were obtained from 1,567 nulliparous pregnant women in three sub-Saharan African sites in Democratic Republic of the Congo (DRC), Kenya, and Zambia. These dried blood spots were subsequently analyzed for *Plasmodium falciparum* infection by qPCR. The prevalence of *P. falciparum* among nulliparous pregnant women in early pregnancy ranged by site: 6.4% in Zambia, 37.6% in Kenya and 63.3% in DRC. Predictors of *P. falciparum* infection in early pregnancy including maternal age, height, body mass index, education, and socioeconomic level will be presented. Women with *P. falciparum* infection in early pregnancy had higher risk of maternal anemia in late pregnancy (crude risk ratio (RR) = 1.66 [95% CI: 1.47, 1.89]), preterm delivery (RR = 1.82 [1.39, 2.40]), perinatal mortality (RR = 1.44 [0.96, 2.17]), and low birth weight (RR = 1.63 [1.22, 2.16]). These results show varying levels of malaria infection in early pregnancy by site. Malaria infection in early pregnancy was associated with higher rates of maternal anemia, preterm delivery, perinatal mortality, and low birth weight. Future steps include assessing predictors of malaria infection in the first trimester (<14 weeks of gestation) and effects of first trimester malaria infection on birth outcomes by each site.

976

ESTIMATING CASES OF SEVERE MALARIA AT THE POPULATION-LEVEL: AN ANALYSIS OF HOUSEHOLD SURVEYS FROM 19 MALARIA ENDEMIC COUNTRIES IN AFRICA

Cameron Taylor[1], Sorrel Namaste[1], Joanna Lowell[2], Johanna Useem[1], Yazoumé Yé[3]

[1]*The DHS Program-ICF, Rockville, MD, United States,* [2]*The DHS Program-Vysnova Partners, Rockville, MD, United States,* [3]*PMI Measure Malaria-ICF, Rockville, MD, United States*

The burden of severe malaria is uncertain at the population level because existing estimates rely exclusively on data from the formal healthcare system. This analysis examined data from 37 Demographic and Health Surveys (DHS) and Malaria Indicator Surveys (MIS) across 19 countries in sub-Saharan Africa collected between 2011 and 2018. The outcome of interest is severe malaria, defined as children age 6-59 months who were rapid diagnostic test (RDT)-positive for malaria with at least one self-reported symptom for severe malaria, including loss of consciousness, rapid breathing, seizures, or severe anemia (hemoglobin levels <5g/dL adjusted for altitude measured using capillary blood). The study includes a weighted descriptive country-level analysis and a multilevel mixed-effects logistic regression model to assess the determinants of severe malaria. Among children RDT-positive for malaria across all surveys, 4.5% (95% CI 4.1-4.8) of children had at least one severe malaria symptom (loss of consciousness, seizures, rapid breathing, or severe anemia), with variations between countries. Severe malaria symptoms were significantly associated with age, residence, wealth quintiles, and survey year. Children in the higher malaria transmission zone were more likely to have symptoms of severe malaria compared to those in the lowest transmission zone; however, these results were not statistically significant. This analysis presents a novel approach of estimating the burden of severe malaria cases in children under age five in malaria endemic countries. The current approach of estimating severe malaria case uses data from the formal healthcare system, which is limited by health care utilization. Having a population level estimate of severe malaria helps further our understanding of the burden and epidemiology of severe malaria.

978

COMPARATIVE GENOMIC ANALYSIS OF RIFIN AND STEVOR VARIANT SURFACE ANTIGENS REVEALS HIGHLY CONSERVED, STRAIN-TRANSCENDENT SEQUENCES AND LIMITED DIVERSITY IN CLINICAL AND REFERENCE ISOLATES

Albert E. Zhou[1], Zalak V. Shah[1], James B. Munro[1], Katie R. Bradwell[1], Emily M. Stucke[1], Kara A. Moser[1], Drissa Coulibaly[1], Mahamadou A. Thera[1], Chanthap Lon[2], Dysoley Lek[3], Stuart D. Tyner[2], David L. Saunders[4], Myaing M. Nyunt[5], Christopher V. Plowe[5], Andrea A. Berry[1], Shannon Takala-Harrison[1], Timothy D. O'Connor[1], David Serre[1], Joana C. Silva[1], Mark A. Travassos[1]

[1]*University of Maryland School of Medicine, Baltimore, MD, United States,* [2]*Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand,* [3]*The National Center for Parasitology, Entomology and Malaria Control, Ministry of Health, Phnom Penh, Cambodia,* [4]*US Army Research Institute of Infectious Diseases, Ft. Detrick, MD, United States,* [5]*Duke Global Health Institute, Duke University, Durham, NC, United States*

The repetitive interspersed family (RIFINs) and subtelomeric variable open reading frame (STEVORs) are antigenically variant gene families in the eukaryotic parasite *Plasmodium falciparum*. Expressed on the surface of infected erythrocytes, these proteins are involved in immune evasion and malaria pathogenesis. Previous analyses of these gene families have relied on targeted amplicons or a few parasite isolates. We performed a comprehensive study of the genetic diversity of the complete RIFIN and STEVOR genomic repertoires in 16 geographically diverse *P. falciparum* strains, using publicly available genome assembly data. We developed a bioinformatic tool based on a set of Hidden Markov Models to automate the identification and classification of RIFIN and STEVOR amino acid sequences. Our program identified RIFINs and STEVORs as well as established PFAM and TIGRFAM tools. In addition, it determined RIFIN subtypes and the number of FHEYDER sequences, motifs associated with severe malaria pathogenesis. We also identified motifs that differentiate RIFIN and STEVOR sequences and increase classification specificity. Serological responses to these motifs may confer cross-reactivity to multiple variants. Comparative analyses of sequence diversity showed that RIFINs and STEVORs share, on average, 45.4% and 60.9% amino acid identity, respectively, between genomes. Amino acid sequence identity for each family did not differ between laboratory and field isolates or by geography. Interestingly, 28 full-length 3D7 RIFIN and 16 full-length 3D7 STEVOR sequences were identical in at least one other isolate. Thirteen 3D7 RIFINs and STEVORs were strain-transcendent, with >75% amino acid sequence identity to homologs in at least seventy-five percent of isolates, suggesting that each of these antigens may have a particular, conserved function that should be explored in future studies. Understanding RIFIN and STEVOR diversity may provide insights into protein function, strain-specific immunity, and malaria vaccinology.

979

EFFICIENT MAPPING OF COMPLEX TRAITS IN THE MALARIA PARASITE *PLASMODIUM FALCIPARUM* USING GENETIC CROSSES AND BULK SEQUENCING

Xue Li[1], Sudhir Kumar[2], Kate Vendrely[3], Marina McDew-White[1], Ann Reyes[1], Katie Button-Simons[3], Abeer Sayeed[1], Lisa Checkley[3], Meseret Haile[2], Spencer Kennedy[2], Ian Cheeseman[1], Stefan Kappe[2], Francois Nosten[4], Michael Ferdig[3], Ashley Vaughan[2], Tim Anderson[1]

[1]*Texas Biomedical Research Institute, San Antonio, TX, United States,* [2]*Seattle Children's Research Institute, Seattle, WA, United States,* [3]*University of Notre Dame, Notre Dame, IN, United States,* [4]*Shoklo Malaria Research Unit, Mae Sot, Thailand*

Classical genetic crosses in malaria parasites requires isolation, genotyping and phenotyping of multiple progeny parasites, which is time consuming, laborious and expensive. We are applying a powerful bulk segregant analysis (BSA) approach that overcomes these problems to identify loci underlying complex traits in the human malaria parasite *Plasmodium falciparum*. This approach combines genetic crosses using humanized mice, with pooled sequencing of progeny populations to measure changes in allele frequency following selection with antimalarial drugs (as one example of selection pressures). Genome regions involved in drug resistance are expected to show differences in allele frequencies between drug treated and untreated progeny populations, and can therefore be used to map drug resistance determinants. Using this BSA approach we mapped: (i) genes underlying parasite growth rate under different *in vitro* culture conditions, and (ii) genes underlying drug resistance. The BSA approach has the potential to robustly identify genes involved in multiple traits including adaptation to vector mosquitoes and selection by the human immune system, that are challenging to examine using classical genetic crosses and analysis of individual progeny.

980

MICROGEOGRAPHIC EPIDEMIOLOGY OF MALARIA PARASITES REVEALED BY MULTIPLEXED, DEEP AMPLICON SEQUENCING AT A SUGAR CANE FACTORY, ETHIOPIA: A HYBRID REPEATED CROSS-SECTIONAL AND PASSIVE CASE DETECTION DESIGN TO STUDY THE IMPACTS OF MIGRATION AND IRRIGATION IN A LOW TRANSMISSION SETTING

Elizabeth Hemming-Schroeder[1], Daibin Zhong[2], Lauren Bradley[2], Delenesaw Yewhalaw[3], James W. Kazura[1], Guiyun Yan[2]

[1]*Case Western Reserve University, Cleveland, OH, United States,* [2]*University of California, Irvine, Irvine, CA, United States,* [3]*Jimma University, Jimma, Ethiopia*

To help combat food insecurity, investments in dams and irrigation in sub-Saharan Africa have increased substantially over the past decade. However, it is not clear to what extent environmental changes from water resource development and associated human migration will affect malaria epidemiology. A hybrid repeated cross-sectional and passive case detection design was used to study the impacts of environmental modifications, changing agricultural practices, and human migration on malaria epidemiology within a sugar cane factory and surrounding communities in southwest Ethiopia. The dynamics of infection complexity, parasite diversity, and population genetic structure were examined by multiplexed deep amplicon sequencing (Illumina Miseq, 2x300 bp paired end reads) at highly polymorphic genes, *pfcpmp* for *Plasmodium falciparum* and *pvmsp1* for *P. vivax*, as well as genotyping of eight microsatellite loci. Travel and migration history information were obtained in questionnaires at the time of specimen collection. Preliminary findings indicate that 80% of *P. falciparum* isolates from people living outside the sugar factory were highly related (shared >90% of alleles) to at least one isolate from someone living within the sugar factory, compared to 82% of isolates within the sugar factory being highly related to another isolate. This result suggests that parasite populations within the factory and surrounding community may be highly interconnected. Further, patients which reported having lived at their current address longer than three years had a statistically significant higher *P. falciparum* multiplicity of infection than those who reported living at their current address less than three years, suggesting that community members which have developed partial immunity to local parasite strains may provide a source of infection for the area. Forthcoming analyses will use newly collected parasite samples to address question of how irrigation schemes and associated migration impacts *P. vivax* epidemiology.

981

PLASMODIUM POPULATION STRUCTURE IN MALARIA LOW TRANSMISSION AREAS OF SOUTH AMERICA: HOW CLOSE ARE WE TO ELIMINATION?

Fabian E. Saenz[1], Claudia A. Vera-Arias[1], Eileen C. Velez-Alvarez[1], Bibiana Salazar[1], Luis E. Castro[2], Javier Gomez-Obando[3], Manuel Calvopiña[4]

[1]*Pontificia Universidad Católica del Ecuador, Quito, Ecuador,* [2]*Ministerio de Salud Publica del Ecuador, Guayaquil, Ecuador,* [3]*Ministerio de Salud Publica del Ecuador, San Lorenzo, Ecuador,* [4]*Universidad de las Américas, Quito, Ecuador*

South America is one of the regions of the world having less malaria incidence. Several countries of this region have been declared capable to eliminate malaria before 2020. One of these countries is Ecuador that saw an impressive decrease in malaria cases of more than 99% between 2001 and 2014. Nevertheless, Ecuador is experiencing a resurgence of falciparum malaria in the northwest coast and vivax malaria in the Amazon of the country. The population structure of *Plasmodium* spp. in both sides of the Andes of Ecuador was analyzed. We used neutral microsatellites (7 for *P. falciparum* and 9 for *P. vivax*) in 107 *P. falciparum* samples from northwest Ecuador (Pacific Coast) from 2013 to 2016, and 118 samples of *P. vivax* from 2013 to 2017 in the coast and Amazon of Ecuador. Our results show that both, *P. falciparum* and *P. vivax* have multiple origins and are more diverse than previously though for a malaria eliminating country. In particular, *P. falciparum* parasites show a low to medium heterocigocity (0.1<He<0.5) with several clonal expansions and *P. vivax* parasites present medium heterocigocity (0.5<He<0.7), no clonal expansions but multiple clone infections. New malaria parasite lineages of *P. falciparum* and *P. vivax* appear to be entering the Ecuador from neighboring countries, which imply difficulties for malaria elimination in the region. Parasite migration corridors in the Pacific coast and the West Amazon of South America maintain the transmission of malaria. Coordinated efforts between countries will be necessary to eliminate the disease in the region.

983

CONTRASTING EPIDEMIOLOGY AND GENETIC VARIATION OF DUFFY NEGATIVE *PLASMODIUM VIVAX* ACROSS AFRICA

Eugenia Lo[1], Daniel Kepple[1], Kareen Pestana[1], Beka Raya[2], Muzamil Mahdi Hamid[3], Delenasaw Yewhalaw[2], Gianluca Russo[4], Giacomo Paganotti[5]

[1]*University of North Carolina Charlotte, Charlotte, NC, United States,* [2]*Jimma University, Jimma, Ethiopia,* [3]*University of Khartoum, Khartoum, Sudan,* [4]*Sapienza University of Rome, Rome, Italy,* [5]*Botswana - University of Pennsylvania Partnership, Gaborone, Botswana*

Plasmodium vivax uses the Duffy antigen/chemokine receptor for entry into human erythrocytes. The proportion of Duffy negativity ranges from 99% in West African to 30-40% in East African countries. There has been ample evidence from several recent studies that showed *P. vivax* can infect Duffy-negative Africans and that they are no longer completely resistant to *P. vivax*. The reports of *P. vivax* in different parts of Africa also raise serious public health concerns of a potential spread across Africa. Compared to *P. falciparum,* several questions regarding the biology and epidemiology of *P. vivax* malaria in Africa are yet unclear. Here, we compare the prevalence, parasitemia, and microsatellite-based variation of *P. vivax* in Duffy negative individuals from West-Central, Southern, and East Africa, as well as to Duffy positive *P. vivax* infections collected in the same areas. We found considerable differences in Duffy-negative *P. vivax* prevalence

among Cameroon, Botswana, and Ethiopia. *Plasmodium vivax* parasite density in Duffy-negative infections is significantly lower than in Duffy-positive infections, regardless of geographical or ethic group differences. Maximum likelihood analyses of the Duffy binding protein sequences indicated that Duffy-negative *P. vivax* isolates were not monophyletic and did not originate from a single common source. Instead, they were found in multiple well-supported clades without clear geographical boundary. By contrast, analyses of five microsatellite loci showed clear genetic structure among the African isolates. The Duffy-negative *P. vivax* from Cameroon, Botswana, and Ethiopia each constituted distinct genetic clusters, different from the Duffy-positive *P. vivax*. *P. vivax* from Ethiopia displays the greatest diversity that constituted admixed clusters resembling both the Duffy-positive and Duffy-negative isolates from Botswana. *P. vivax* from Cameroon forms distinct lineages. These findings help clarify the genetic origin and spreading pathways of *P. vivax* in Africa. This information will contribute to our limited knowledge of *P. vivax* epidemiology and biology in Africa.

984

COMPARATIVE *PLASMODIUM FALCIPARUM* POPULATION GENETIC STRUCTURES IN COGNATE HUMAN AND MOSQUITO HOSTS IN WESTERN KENYA

Kelsey M. Sumner[1], David Rasmussen[2], Elizabeth Freedman[3], Lucy Abel[4], Andrew Obala[5], Steven R. Meshnick[1], Wendy Prudhomme-O'Meara[3], Steve M. Taylor[3]

[1]*University of North Carolina at Chapel Hill, Chapel Hill, NC, United States,* [2]*North Carolina State University, Raleigh, NC, United States,* [3]*Duke University, Durham, NC, United States,* [4]*Moi Teaching and Referral Hospital, Eldoret, Kenya,* [5]*Moi University, Eldoret, Kenya*

Plasmodium falciparum genetic diversity undermines malaria control by enabling parasite adaptation to changing fitness landscapes. A clearer understanding of the factors which shape parasite genetic diversity could enable directed approaches to limiting diversity and enhancing control measures. Transitions between human and mosquito hosts could shape diversity and represent a genetic bottleneck, as the transitions are executed by minimal numbers of parasites. We investigated this possibility using 973 samples of human *P. falciparum* infections, 123 infected *Anopheles* mosquito heads, and 185 infected *Anopheles* mosquito abdomens collected in sentinel households during a 14-month longitudinal study in Webuye, Kenya. In each human or mosquito infection, we cataloged unique genotypes (represented by haplotypes) created by amplicon deep sequencing of a polymorphic parasite gene target (*pfcsp).* After sub-setting infections into time- and space-restricted analytic populations, we used population genetic indices and models to compare parasite populations between compartments. Overall, we observed 298 *pfcsp* haplotypes. The mean (standard deviation) multiplicity of infection was 4.2 (3.7) in human infections, 6.4 (3.4) in infected mosquito abdomens, and 5.7 (3.2) in infected mosquito heads. In human infections, we observed 155 haplotypes with 91/155 (58.7%) found only within human infections. Similarly, we observed 143 haplotypes in infected mosquito abdomens with 76/143 (53.1%) solely in mosquito abdomens. For infected mosquito heads, we observed less haplotypes than the other two groups (N=118) and 55/118 (46.6%) were only in mosquito heads. Less haplotypes were found in the mosquito abdomens and heads compared to the human infections. Longitudinal multi-level modeling results indicated that the number of haplotypes observed over time was lower in the mosquito abdomens and heads compared to the humans. These results are consistent with a reduction in genetic diversity in the mosquito stages of *P. falciparum* in a highly endemic setting, and suggest value in targeting these stages for surveillance and future interventions.

986

USING THE M-LOCUS GENE *NIX* FOR *AEDES AEGYPTI* SPERM QUANTIFICATION

Miguel Ángel Toro-Londoño, Frank W. Avila

Max Planck Tandem Group in Mosquito Reproductive Biology - Universidad de Antioquia, Medellín, Colombia

Aedes aegypti mosquitoes are responsible for the transmission of viruses that impact human health including the dengue, Zika and chikungunya viruses. Control of *Ae. aegypti* mosquitoes relies primarily on insecticide use or on contemporary control methods that utilize transgenic or *Wolbachia* infected adults to suppress or replace native populations. Despite the potential of exploiting reproduction for the purpose mosquito population control, many processes that mediate mosquito reproduction remain to be examined. One aspect of *Aedes* reproduction requiring further elucidation is the storage of sperm in mated females. Sperm storage is a universal process in animals with internal fertilization, and a process required for the fertility of a mating pair. Although methods to assess *Aedes* sperm storage parameters have been described, these methods are laborious, restricting their use to assess this essential reproductive process. Here, we developed a molecular approach to assess sperm transfer and overall sperm storage in *Ae. aegypti* females by determining the copy number of *Nix*, the dominant male-determining factor (M factor) that resides on the male-specific M-locus of chromosome 1; inheritance of *Nix* results in male development. We first developed a strategy to amplify *Nix* from mated females using conventional PCR. The *Nix* amplicon was then used to assemble a plasmid calibrator to molecularly "count" 1) sperm transferred from males to females, and 2) total sperm stored in the spermathecae of the female reproductive tract by qPCR. A manual counting strategy was used to validate our molecular assay. This approach has the potential for use in other *Aedes* species, and with minor changes using the *Yob* gene, in Anopheline species.

987

THE RAPID DETECTION OF ZIKA, DENGUE AND CHIKUNGUNYA VIRUSES IN *AEDES AEGYPTI* MOSQUITOES TO PRODUCE A RAPID RESPONSE VECTOR CONTROL

Gabriela A. Garcia[1], Lilha M. Santos[1], Mariana R. David[1], Marcio G. Pavan[1], Maggy Sikulu-Lord[2], Anton Lord[3], Rafael Maciel-de-Freitas[1]

[1]*Oswaldo Cruz Foundation, Rio de Janeiro, Brazil,* [2]*School of Public Health, University of Queensland, Brisbane, Australia,* [3]*QIMR Berghofer Medical Research Institute / School of Public Health, University of Queensland, Brisbane, Australia*

Dengue (DENV), Zika (ZIKV) and Chikungunya (CHIKV) are transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes in the tropical regions of the world. The transmission of these viruses to humans could be significantly reduced with their timely detection in the mosquito vector. This allows timely implementation of vector control to stop a future outbreak. Herein, we tested the near infrared spectroscopy (NIRS) technique, for rapid and cost-effective detection of arboviruses in *A. aegypti* mosquitoes. NIRS is a rapid and cost effective technique that involves a 3-5 second interaction of a beam of light with samples. We performed experiments using mosquitoes infected with currently circulating strains of Brazilian ZIKV, DENV and CHIKV and uninfected fresh and mosquitoes left in BG-sentinel traps for 0-7 days post death. Spectra were collected using NIR spectrometer (LabSpec 4 i NIR spectrometer, Model 135325 Rev B, ASD Inc., Malvern Panalytical) at 4-, 7-, 10- and 14- days post-infection (dpi) for infected and uninfected cohorts to predict the presence or absence of arboviruses. Partial least square (PLS) was used to develop infection prediction models. Models were validated on a sub-sample of mosquitoes that were excluded in the model. The accuracy of NIRS for detecting arbovirus in fresh *A. aegypti* females ranged from 94.2 to 100% for ZIKV and 74-100% for DENV. Moreover, NIRS could differentiate mosquitoes co-infected with ZIKV/DENV with a predictive accuracy of 96.76%. Models for predicting infection in mosquitoes left in

BG-sentinel traps for a period of 7 days ranged from 95.6% to 99.8% for CHIKV and 90.6% to 95.1% for ZIKV depending on the day post death the mosquitoes were scanned. Additional research is needed to investigate if similar accuracy might be observed in field caught mosquitoes naturally infected with those arboviruses. However, these preliminary results are promising and indicate that NIRS could potentially be used in the field to scan thousands of samples to test for infectivity of these viruses at a relatively low cost compared to existing tools.

988

INDICES OF HUMAN EXPOSURE TO *ANOPHELES* BITES IN CENTRAL AND SOUTHERN MALAWI

Evelyn A. Olanga[1], Nellie C. Kaunde[1], Eggrey A. Kambewa[1], Judith S. Banda[1], Christopher M. Jones[2], Lisa Reimer[3], Charles Wondji[4], Philip McCall[3], Hilary Ranson[3], Themba Mzilahowa[1]

[1]*Malaria Alert Centre of the College of Medicine, Malawi, Blantyre, Malawi,* [2]*Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre, Malawi,* [3]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [4]*Centre for Research in Infectious Diseases, Yaounde, Cameroon*

Treated bednets are the cornerstone of malaria vector control in Malawi. In 2018, Malawi distributed approximately 8 million and 2 million pyrethroid-only and piperonyl butoxide (PBO) nets, respectively. There is a need to determine whether bednets have a high protective efficacy, taking into account human and mosquito behaviour. Mosquito and human behaviour surveys were undertaken during the rainy season between December 2019 and March 2020 in Ntwana and Kamwendo villages in Chikwawa and Dedza districts, respectively. Mosquito biting activity was estimated by human landing catches for four nights per month. In March a questionnaire was administered to residents to determine usage of bednets and time spent indoors and in bed during the night. Indices of human exposure to mosquito bites that occur while people are indoors and asleep and protection against bites were calculated based on a published mathematical model. A total of 9,014 anophelines were captured of which the proportion caught in Dedza (81%; n=7,356) was four times higher than Chikwawa (19%; n=1,658). *Anopheles arabiensis* (91%) was the major vector in Dedza and *An. funestus* (99%) in Chikwawa. The biting activity of *An. arabiensis* was higher outdoors than indoors (OR=1.04, CI=[1.046-1.048], P<0.001). *An. funestus* was most likely to bite indoors compared to outdoors (OR=1.12, CI=[1.11-1.13], P<0.001). Taking into account human behaviour, exposure to *An. funestus* bites for non-bednet users mainly occurred indoors (96% in Chikwawa and 97% in Dedza). However, exposure to *An. arabiensis* bites indoors and when people were asleep was <90% in both districts. Treated bednets were estimated to offer >70% protection against *Anopheles* bites for users (78% in Chikwawa and 74% in Dedza). The findings show that transmission primarily occurs indoors at night in Chikwawa. Despite higher outdoor biting activity in Dedza, transmission still occurs indoors at night when people are asleep. Intensive use of treated bednets should be prioritized in Chikwawa in areas where *An. funestus* is prevalent, while interventions that target outdoor biting mosquitoes sought to supplement bednets in Dedza.

989

RAPID INDUCTION OF APOPTOSIS IN *AEDES AEGYPTI* MIDGUTS FOLLOWING DENGUE-2 OR ZIKA VIRUS INFECTION

Jasmine Blue Ayers, Rhoel Dinglasan, Lei Zhou

University of Florida, Gainesville, FL, United States

Dengue or Zika virus transmission requires infection of the mosquito midgut, followed by spread to the salivary glands so virus can be injected into the next human host during feeding. Certain strains of *Aedes aegypti* are refractory to arbovirus infection, i.e., arboviruses fail to establish a midgut infection. Within 2 hours of ingesting a dengue-2 or Zika virus infected blood meal, we observe caspase activation and DNA fragmentation in midgut epithelial cells of lab-adapted *Ae. aegypti* strains (Orlando [ORL] and MOYO-R), indicating a programmed cell death response. Acute inhibition of apoptosis by cofeeding human alpha-1 antitrypsin with a virus-infected bloodmeal results in increased viral genome replication at 48 hours and midgut viral titers at 7 days post-infection. These data suggest that a rapid induction of apoptosis (RIA) may aid in resisting virus establishment in the midgut. We hypothesize that RIA is an intrinsic organismal defense mechanism to clear infected cells via apoptosis before viral replication can occur. However, the means by which virus exposure triggers cell death in the midgut within hours of feeding remains unclear. To gain deeper insight, we used single molecule RNA fluorescence *in situ* hybridization (smRNA FISH) to track whether dying cells are infected by virus at these early time points post-infection, and immunofluorescence assays for markers of cell type such as centrin and phosphorylated histone 3 to differentiate stem cells, enteroblasts, and enterocytes, and describe which subpopulations of midgut cells are involved in the RIA response. Finally, to parse whether RIA is triggered by viral adhesion or viral gene products, we compared infection with replication competent virus to exposure to ultraviolet-C inactivated virus. Understanding the cellular response to DENV and ZIKV infection in the midgut will aid in understanding the underlying mechanism of *Ae. aegypti* refractoriness to these viruses, which is a topic of great epidemiological interest, both for predicting the ability of local populations to transmit them and for engineering mosquito strains that can resist infection.

990

USING MULTI-SCALE REMOTELY SENSED DATA TO UNDERSTAND SPATIO-TEMPORAL PATTERNS IN MALARIA RISK IN CENTRAL MALAWI: HOW HIGH DO WE NEED TO FLY?

Patrick K. Kalonde[1], Christopher M. Jones[1], Kennedy K. Zembere[1], Michelle C. Stanton[2]

[1]*Malawi-Liverpool-Wellcome Trust, Blantyre, Malawi,* [2]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom*

Remotely sensed data are commonly used to gain information about the environment and to subsequently link this information to observed patterns in disease risk. For example, for vector-borne diseases such as malaria, maps of vector habitat can be used to identify human populations most at risk of being bitten by an infected mosquito. While imagery captured by satellite, orbiting hundreds of kilometres above the earth, are most commonly used for disease risk mapping tasks, very high-resolution imagery (<10cm) captured by drones flying at altitudes of approximately 100m is becoming increasingly available. The purpose of our study was to identify patterns in malaria risk using data captured using drones and satellites, and to determine the circumstances by which the benefits of drone imagery outweigh the additional expenses (time, costs, computer processing requirements etc) incurred when using this technology. The study took place in Kasungu district, Central Malawi in an area designated as a 'drone testing corridor' by the government of Malawi. Images were captured by drones to identify permanent and temporary surface water, and larval surveys were simultaneously undertaken to identify water in which mosquitoes were laying their eggs. Machine learning techniques were applied to the imagery to produce a larval habitat risk surface. Satellite imagery was then obtained for a similar time period, and further classified to identify surface water. The relationship between these risk surfaces and clinical malaria were explored using routinely collected data extracted from the national health management information system (HMIS). Malaria risk information obtained from both sources of imagery were compared and evaluated in relation to their potential to provide information that could be used to guide malaria control activities. We conclude that while imagery captured by drones has the potential to provide highly detailed, contemporary information on the disease risk landscape, the additional efforts involved in obtaining this information are only worthwhile if planned interventions operate at an equally high spatial and temporal scale.

992

ALTERNATIVE TREATMENT STRATEGIES IN ACCELERATING ONCHOCERCIASIS ELIMINATION IN THE MASSANGAM HEALTH DISTRICT IN CAMEROON

Kareen Atekem[1], Ruth Dixon[2], Rogers Nditanchou[1], Benjamin Biholong[3], Joseph Oye[1], Hugues Nana Djeunga[4], Philippe Nwane[4], Franklin Ayisi[3], Daniel Boakye[5], Joseph Kamgno[4], Elena Schmidt[2], Laura Senyonjo[2]

[1]*Sightsavers, Yaounde, Cameroon,* [2]*Sightsavers, Haywards Heath, United Kingdom,* [3]*National Onchocerciasis Control Program, Ministry of Health, Yaounde, Cameroon,* [4]*Centre for Research on Filariasis and other Tropical Diseases (CRFilMT), Yaounde, Cameroon,* [5]*Parasitology Department, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Accra, Ghana*

Studies in Mali and Senegal demonstrated that 15-17 years of annual community-directed treatment with Ivermectin (CDTI) can interrupt onchocerciasis transmission. However, in Massangam health district (MHD), Cameroon, after more than 20 years of CDTI, transmission remains persistently high. We implemented and evaluated alternative treatment strategies (ATS) including biannual IVM, test and treatment with doxycycline and ground larviciding, with aim of reducing transmission and accelerating progress towards elimination in this district. Initially, parasitological, entomological and breeding site survey were conducted in 2015, delineating a focus of high transmission and identifying 3 communities with high microfilaria (mf) prevalence. Individuals in these communities were screened for mf yearly for a period of 2 years and positives treated each year with doxycycline 100mg daily for 5 weeks. In addition, 14 surrounding communities were given biannual IVM. Temephos-based applications were performed once a week during 10 consecutive weeks on *Simulium* breeding sites previously identified along rivers Mbam and Nja. Parasitological and entomological assessments were conducted after 2 years of implementation and findings compared with 2015 baseline. This approach recorded a screening coverage of 54.3% and 56.7% (2017, 2018) of population eligible for doxycycline treatment in the 3 communities. In the surrounding communities, IVM coverage of 72.2% and 81.1% was recorded for 2017 and 2018 respectively. Microfilarial prevalence in the three communities reduced significantly from 32.6% (95% CI 24.4-40.8) to 13.2% (8.9% - 17.5%) ($P < 0.001$) after implementation. Regarding entomological indices, both infection and infective rates of flies remained similar between baseline and end-line (0.25% - 0.26% and 0.06% - 0.11% respectively). This study revealed that the strategy used is a feasible and effective alternative strategy which should be considered in areas where transmission is sustained throughout long term uninterrupted MDA with IVM. Cost study is ongoing and will provide more insights regarding scalability of this strategy.

993

COMPARING POST-MDA COVERAGE SURVEY DATA FOR TRACHOMA, ONCHOCERCIASIS AND LYMPHATIC FILARIASIS WITH REPORTED DATA AMONG 14 DISTRICT COUNCILS IN TANZANIA

Veronica Kabona[1], Denis Kailembo[1], Gerald Robi[1], Oscar Kaitaba[2], Kerry Dobies[3], Mary Linehan[3], Abdel Direny[3], Josh West[4], Benjamin Crookston[4]

[1]*IMA World Health, Dar Es Salaam, United Republic of Tanzania,* [2]*Ministry of Health, Dar Es Salaam, United Republic of Tanzania,* [3]*IMA World Health, Washington, DC, United States,* [4]*Bringham Young University, Provo, UT, United States*

All districts of Tanzania are endemic for ≥2 preventive chemotherapy neglected tropical diseases (NTDs). Mass drug administration (MDA) interventions in Tanzania have interrupted transmission of lymphatic filariasis (LF) in 105 out of 120 district councils and Trachoma in 65 out of 71. In October 2019, the Tanzania NTD Control Program (TZNTDCP) conducted a post-MDA coverage evaluation survey (CES) in 14 councils to compare reported coverage for Trachoma, Onchocerciasis and LF data from community drug distributors (CDDs), with data collected from the survey. The purpose was to first, estimate concordance between the two datasets, and second, identify reasons for not participating in MDA. The CES was a cross-sectional survey following the World Health Organization (WHO) guidelines using probability proportional to estimated size. Councils were selected if they had failed a disease specific assessment (DSA) at least once by 2019 for LF or Trachoma, and those with the change of MDA strategy to twice MDA yearly for Onchocerciasis. In two councils (14.2%) there was higher coverage in the CES results when compared to the one reported by CDDs (81.8% vs 70.1% and 78.8% vs 71%). WHO set the minimum coverage for MDA at 65% for LF and 80% for Onchocerciasis and Trachoma. Successful MDA was found in 1 out of 4 councils (25%) for Trachoma, 1 out of 3 councils (33.3%) for Onchocerciasis and in 7 out of 7 the councils (100%) for LF when comparing the CES validated coverage with the WHO targets. This contradicts reported MDA coverage by CDDs that shows 12 out of 14 surveyed councils achieved the recommended MDA coverage. The CES also revealed that absenteeism was the main reason that people did not participate in MDA (38.1% for Trachoma and 36.3% for LF and Onchocerciasis), followed by drug distributors did not arrive (22.4% for Trachoma and 16.7% for LF and Onchocerciasis). New strategies such as strengthening advocacy and social mobilization as well as improving CDDs training, based on the CES results, will be implemented by the TZNTDCP in councils which had low MDA coverage validated by the CES, to improve coverage and subsequently eliminate NTDs in the country as a whole.

995

CHALLENGES OF TRANSMISSION ASSESSMENT SURVEYS (TAS) FOR DETERMINING TRANSMISSION INTERRUPTION FOR LYMPHATIC FILARIASIS IN EAST NEW BRITAIN PROVINCE, PAPUA NEW GUINEA

Krufinta Bun[1], Michael Payne[1], Daniel Tisch[1], Catherine Bjerum[1], Benedict Mode[2], Moses Laman[3], Melinda Susapu[4], Makoto Sekihara[5], Peter J. Diggle[6], Emanuele Giorgi[6], Leanne Robinson[7], Gary Weil[8], Christopher L. King[1]

[1]*Case Western Reserve University, Cleveland, OH, United States,* [2]*East New Britain Provincial Health Department, Kokopo, Papua New Guinea,* [3]*Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea,* [4]*National NTD Program, Ministry of Health, Port Moresby, Papua New Guinea,* [5]*Japan International Cooperation Agency (JAICA), Tokyo, Japan,* [6]*Lancaster University, Lancaster, United Kingdom,* [7]*Burnet Institute, Melbourne, Australia,* [8]*Washington University, St. Louis, MO, United States*

East New Britain Province (ENBP) is located in Papua New Guinea with a population of 375,000 without prior province-wide mass drug administration (MDA) for lymphatic filariasis (LF). Prior convenience sampling showed ENBP to be endemic for LF. In 2019, a survey of 30 randomly selected villages (population proportional sampling) along with 5 purposely selected village suspected to have high LF infection rates was conducted across 4 districts prior to MDA with ivermectin, diethylcarbamazine and albendazole (IDA). In each village, ~50 participants aged 6-9 years in randomly selected households and a similar number of individuals >10 years were tested for filarial antigen positivity using filaria test strip (FTS) and for microfilaria. Baseline FTS prevalence was: 0.7% (CI:0.22-1.2) for 6-9 years (N=1121); 1.9% (CI: 0.61 - 3.2) for 10-17 years (N=411); 4.2% (CI:2.9 - 5.4) for > 18 years (N=1079); and 4.6% (CI: 2.5-6.7) for >18-year-old males. No MF positives were found among 6-9 years, whereas those > 18 years had 1.2% (CI:0.4 -1.3%) microfilarial positivity. Because of these initial low infection rates, 19 additional communities suspected of having higher infection were evaluated and showed FTS infection prevalence of 3.6% (CI: 2.3-5.0) for 6-9 years, 6.2% (CI: 3.1-9.2) for 10-17 years, 16.7% (CI:13.7-19.4) for >18 years, and 20.9% (CI:15.5-26.2) for >18-year-old males. We used a geostatistical model to map FTS prevalence across the study areas. The resulting risk maps showed a higher risk in rural areas. These results highlight the challenge of transmission assessment survey (TAS) protocols using population proportional sampling that will oversample urban areas with lower risk of LF transmission in areas where Anopheline mosquitoes are the vector. Alternative TAS sampling

strategies need to be considered such as focusing on sampling adults with the highest risk of infection, using LF risk mapping and investigating indices such as vegetation cover, altitude and/or social economic status after MDA. The results highlight an opportunity to revisit and evaluate the TAS sampling guidelines for LF elimination in endemic countries.

996

MONITORING IMPACT OF MASS DRUG ADMINISTRATION USING A 3-DRUG REGIMEN ON LYMPHATIC FILARIASIS IN AMERICAN SAMOA

Tara A. Brant[1], Aifili J. Tufa[2], Fara Utu[2], Lynette Suiaunoa-Scanlan[3], June Vaifanua-Leo[2], Loretta S. Lees[2], Benjamin Sili[2], Rebecca J. Chancey[1], Marisa A. Hast[1], Keri L. Robinson[1], Emily A. Dodd[1], Janet Camacho[4], Emi Chutaro[4], Kimberly Y. Won[1], Motusa T. Nua[2]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*American Samoa Department of Health, Pago Pago, American Samoa,* [3]*Pacific Island Health Officers' Association, Pago Pago, American Samoa,* [4]*Pacific Island Health Officers' Association, Honolulu, HI, United States*

The standard strategy for lymphatic filariasis (LF) elimination is annual mass drug administration (MDA) using a combination of albendazole and either diethylcarbamazine (DEC) or ivermectin. Recently, a 3-drug regimen of ivermectin, DEC, and albendazole (IDA) was introduced that may accelerate LF elimination. From 2000-2006, American Samoa conducted 7 rounds of standard MDA; periodic assessments found LF prevalence below the 1% threshold requiring MDA. However, results from a 2016 survey found 6.2% LF prevalence, signaling the need to resume MDA. In 2018, the American Samoa Department of Health (DOH) conducted IDA MDA in the territory, with round two completed in October 2019. To monitor progress, DOH conducted coverage surveys in February 2019 and 2020 following each MDA. In Jul-Aug 2019, DOH conducted an impact assessment among 2,081 participants aged ≥5 years in all 68 villages on Tutuila and Aunu'u islands. Finger stick blood samples were collected to assess circulating filarial antigen (CFA) by filariasis test strip, and CFA positive participants were tested for microfilaremia (Mf). All CFA and Mf positive participants were treated with IDA. Mf positive participants were examined 7 days after treatment for clearance of Mf. Drug coverage in 2018 and 2019 was 72.7% (95% confidence interval (CI): 66.5%-78.0%) and 76.7% (CI: 71.9%-81.0%), respectively. After one round of IDA, age-adjusted CFA prevalence was 2.7%. Eight of 47 (17.0%) CFA positive participants were Mf positive. Seven of 8 Mf positive participants were followed up one week after treatment and no Mf were detectable. CFA positivity was higher among participants ≥10 years (2.7%) than 5-9 years (1.1%). Drug coverage for CFA positive participants was lower (65.9%) than the total population (72.7%, p=0.36). CFA positivity was higher among males (4.8%) than females (1.0%, p<0.001), but coverage was similar between sexes. Findings of drug coverage exceeding the minimum recommendation of 65% and declines in LF prevalence suggest progress towards elimination. However, it may be necessary to target certain residents (e.g., men) in future MDAs to achieve elimination in American Samoa.

997

SEGMENTING IMPLEMENTATION UNITS (IUS) DURING PRE-TAS IN HAITI TO STRENGTHEN MASS DRUG ADMINISTRATION (MDA) IN CONFIRMED HOTSPOTS

Carl Renand Fayette[1], Alain Javel[1], Eurica Denis[1], Paul-Emile Dalexis[1], Marc-Aurele Telfort[2], Ellen Knowles[3], Abdel Direny[3], Mary Linehan[3], Josh West[4], Benjamin Crookston[4]

[1]*IMA World Health, Port au Prince, Haiti,* [2]*Ministry of Health and Population, Port au Prince, Haiti,* [3]*IMA World Health, Washington, DC, United States,* [4]*Brigham Young University, Provo, UT, United States*

Persistent efforts to eliminate lymphatic filariasis (LF) in Haiti over the past two decades have resulted in large reductions in prevalence nationwide. As a result, 121 out of 140 implementation units (IUs) in the country no longer require mass drug administration (MDA). Despite this progress, 19 IUs have on-going transmission after having completed at least nine rounds of MDA. These IUs have had high baseline prevalence (antigenemia: 10-45%) and are demographically diverse. The Haiti Neglected Tropical Disease Control Program (HNTDCP), with support from its partners, created an adapted approach during sentinel and spot-check site surveys. While these surveys are usually in one sentinel and one spot-check site per IU, the program segmented 4 IUs into 2-3 smaller, sub-units based on their suspected risk. The results showed an age range of antigen-positives between 25-68 years old in all 4 IUs; 1.6% of females in all 4 IUs were positive while 3.6% of males were positive. The results also revealed that, rather than complete failures in IUs when considered as a whole, 33% of the sub-units now meet the World Health Organization (WHO) criteria for eligibility to implement a transmission assessment survey (TAS) to determine if MDA can be stopped (Ag<2% in sentinel and spot-check sites). With limited resources, segmenting IUs in Haiti can be a solution for tailoring MDA strategies for hard-to-reach and non-compliant populations, and to apply resources efficiently, where needed. With this approach, the HNTDCP will be on track with the new WHO roadmap, which aims to eliminate LF as a public health problem by 2030.

998

INTERRUPTION OF ONCHOCERCIASIS TRANSMISSION IN NIGER

Salissou Adamou[1], Yaobi Zhang[2], Yaye Youssouf[3], Daniel Boakye[4], Charles Mackenzie[4], Jamie Tallant[4], Sashi Leff[4], Sara Laskowski[4]

[1]*Ministry of Health, Niamey, Niger,* [2]*Helen Keller International, London, United Kingdom,* [3]*Helen Keller International, Niamey, Niger,* [4]*END Fund, New York, NY, United States*

Niger was one of the countries of the former Onchocerciasis Control Programme in West Africa (OCP) with onchocerciasis endemic in five health districts (Tera, Say, Kollo, Birni Gaoure and Gaya). The endemic area is considered as a single transmission zone with five main Rivers (Niger, Diamongou, Tapoa, Sirba and Gourouba); baseline infection prevalence in 1976/1977 ranged from 44.2 to 71.2%. Onchocerciasis control in Niger was through vector control alone from 1976-1985; this was integrated with ivermectin for those found positive and their families from 1986-2002. Although MDA with ivermectin was not done specifically for onchocerciasis in Niger, all endemic districts are co-endemic with lymphatic filariasis and have benefited from ivermectin MDA for LF from 2003. At the end of the OCP in 2002, onchocerciasis prevalence (skin snip) was zero throughout the onchocerciasis endemic area; vector infection rate was also zero. To confirm that transmission has indeed been interrupted on the criteria of WHO guidelines, entomological evaluations were done in 2014 (7 sites), 2015 (14 sites) and 2016 (14 sites). The number of *S. damnosum* s.l. females subjected to O-150 poolscreening PCR for each of these years were 10,405; 26,513 and 126,098. Infectivity rates were zero at all sites with upper 95% confidence limits of 0.18%, 0.07% and 0.01% for each of these years. Although in 2014 and 2015, no infection was found in at any site, the number of flies collected at each site were generally well below the 6,000 expected. In 2016 all sites had above the expected 6,000 with the minimum being 6,244. Epidemiological assessment of 15,600 children below 10 years of age using OV-16 serology gave 0.03%. The entomological and epidemiological data, show that onchocerciasis transmission has been interrupted and maybe eliminated in the traditional onchocerciasis endemic regions of Niger. To achieve a country-wide verification of onchocerciasis elimination, Niger is currently evaluating other areas not known for onchocerciasis to ensure that all areas of the country are considered in this important achievement for onchocerciasis elimination in Africa.

1000

COMPARISON OF PERFORMANCE OF CRISPR-CAS9 VERSUS -CAS12A RIBONUCLEOPROTEIN COMPLEXES IN EDITING THE *SCHISTOSOMA MANSONI* OMEGA-1 GENE

Wannaporn Ittiprasert

George Washington University, Washington, DC, United States

Delivery of CRISPR reagents into the cells of the multicellular parasite *Schistosoma mansoni* in the form of ribonucleoprotein (RNP) complexes can enable immediate and permanent programmed gene editing and obviate concerns with CRISPR reagent longevity or integration in the host cell genome. Programmed CRISPR-editing using RNP-based delivery can precede analysis of mutated target regions that rely on cloning and vector construction steps. Previously, we |successfully deployed a lentiviral-delivered CRISPR/Cas9 approach to edit (both knock-out and knock-in) the schistosome omega-1 (ω1) gene in parasite eggs. Gene editing efficiency using the *Streptococcus pyogenes* Cas9 nuclease was modest at best. To improve on this outcome, we hypothesized that Cas12a (Cfp1) from *Acidaminococcus* sp. That, following cleavage leaves sticky ends at the double strand break (DSB), may enhance efficiency of editing of the ω1 gene in comparison with Cas9, which catalyzes blunt ended DSBs. CRISPR/Cas12a recognizes the TTTV protospacer adjacent motif (PAM) to cleave the target gene downstream of its PAM, whereas the Cas9 recognizes a PAM of NGG. Gene editing in mammalian cell lines had shown that CRISPR/Cas12a performed with higher efficiency and precision to knock-in donor transgenes by homology directed-repair (HDR) in comparison to Cas9. In like fashion, we observed that, based on transcript reduction, Cas12a performed better than Cas9 following delivery as RNPs. In particular, following delivery of RNPs to eggs *in vitro,* transcripts encoding ω1 were undetectable by RT-PCR for Cas12a. By contrast, Cas9 reduced ω1 transcripts by ~70%. The insertion-deletion (INDEL) profiles will be discussed along with profiles of transgene knock-in events resulting from HDR of Cas9 *vs*. Cas12a cleavages of the ω1 locus.

1001

CRISPR-MEDIATED TRANSFECTION OF *BRUGIA MALAYI*

Canhui Liu[1], Alexandra Grote[2], Elodie Ghedin[3], **Thomas R. Unnasch**[1]

[1]*University of South Florida, Tampa, FL, United States,* [2]*Broad Institute of MIT and Harvard, Cambridge, MA, United States,* [3]*New York University, New York City, NY, United States*

The human filarial parasites have lagged in the application of reverse genetic technologies due to the difficult biology of these organisms. Recently, a co-culture system was developed that permitted the infective larval stage of *Brugia malayi* to be transfected and efficiently develop to fecund adults. This was exploited to develop a *piggyBac* transposon-based toolkit that can be used to produce parasites with transgene sequences stably integrated into the parasite genome. However, the *piggyBac* system has generally been supplanted by Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) based technology, which allows precise editing of a genome. Here we report adapting the *piggyBac* mediated transfection system of *B. malayi* for CRISPR mediated knock-in insertion into the parasite genome. Suitable CRISPR insertion sites were identified in intergenic regions of the *B. malayi* genome. A dual reporter *piggybac* vector was modified, replacing the *piggyBac* inverted terminal repeat regions with sequences flanking the insertion site. *B. malayi* molting L3 were transfected with a synthetic guide RNA, the modified plasmid and the CAS9 nuclease. The transfected parasites were implanted into gerbils and allowed to develop into adults. Progeny microfilariae were recovered and screened for expression of a secreted luciferase reporter encoded in the plasmid. Approximately 3% of the microfilariae were found to secrete luciferase; all contained the transgenic sequences inserted at the expected location in the parasite genome. Using an adaptor mediated PCR assay, transgenic microfilariae were examined for the presence of off target insertions; no off target insertions were found. These results demonstrate that CRISPR can be used to modify the genome of *B. malayi*, opening the way to precisely edit the genome of this important human filarial parasite.

1002

CHARACTERIZATION OF THE CHEMOSENSORY PATHWAY OF FILARIAL WORMS

Nicolas J. Wheeler, Zachary W. Heimark, Paul M. Airs, Alexis Mann, Lyric C. Bartholomay, Mostafa Zamanian

University of Wisconsin-Madison, Madison, WI, United States

Helminth larvae of diverse life histories perform migrations to reach their final destination, where they can reproduce and complete their life cycle. The migrations performed by helminths are some of the most fascinating elements of parasitology, and often the first thing a novice parasitologist must learn about their organism of interest. Unfortunately, very little is known about the genetic and molecular basis for migration for the vast majority of helminth species, especially those that are vector-borne like filarial parasitic nematodes. While it is well known that nematodes contain a conserved set of amphid organs used to sense the environment during migration, new genomic data sources for the first time facilitate the identification of conserved sensory signaling pathway effectors in nematodes. We identified and annotated over 30,000 putative chemoreceptors in free-living and parasitic nematodes and discovered a correlation between the number of chemoreceptors in a given species and the presence and extent of free-living terrestrial life stages experienced by that species. Filarial nematode genomes contain a severely restricted complement of chemoreceptors, which are expressed in stage and tissue-specific patterns that correlate with migratory timepoints. Chemical perturbation and functional genomics in *Brugia* demonstrate that two ion channels, OSM-9 and TAX-4, function during the parasitization of mosquito vectors and are necessary for *in vitro* chemotaxis of infective larvae toward a host-derived cue. Finally, *B. malayi* OSM-9 and TAX-4 can partially rescue sensory defects in the model nematode *C. elegans*, demonstrating some functional conservation in diverse species across the phylum. These data provide a broad foundation for the study of sensory biology in nematodes and motivate further research of sensory receptors and pathways as drug targets in filarial nematodes.

1003

ATTENUATED VIRULENCE FOLLOWING PROGRAMMED GENOME EDITING OF THE CARCINOGENIC LIVER FLUKE, *OPISTHORCHIS VIVERRINI*

Thewarach Laha[1], Wannaporn Ittiprasert[2], Patpicha Arunsan[1], Sujittra Chaiyadet[1], Sirikachorn Tangkawatttana[1], Michael J. Smout[3], Alex Loukas[3], **Paul J. Brindley**[2]

[1]*Khon Kaen University, Khon Kaen, Thailand,* [2]*The George Washington University, Washington DC, DC, United States,* [3]*James Cook University, Cairns, Australia*

Infection with the food-borne liver fluke *Opisthorchis viverrini* is the principal risk factor for cholangiocarcinoma (CCA) in the Lower Mekong River Basin countries including Thailand, Lao PDR, Vietnam, Myanmar, and Cambodia. We have exploited this link to explore the role of the secreted growth factor termed liver fluke granulin (*Ov*-GRN-1) and other fluke antigens including tetraspanin-2 (*Ov*-TSP-2) in pre-malignant lesions by undertaking programmed CRISPR/Cas9 gene knockout. Analysis by RT-PCR, immuno-blots, and nucleotide sequencing of amplicon libraries spanning programmed mutations confirmed the activity of CRISPR gene editing in this parasite, confirmed Cas9-catalyzed mutations in target genes, and revealed the rapid depletion of transcripts and the targeted proteins. Whereas gene-edited parasites colonized the biliary tract of hamsters and developed into adult flukes, the infection-induced less disease as evidenced by attenuated biliary hyperplasia and fibrosis. Follow-up studies in hamsters both treated with a sub-carcinogenic dose of dimethylnitrosamine and infected with gene-edited flukes revealed the delayed onset of cholangiocarcinogenesis. The programmed mutations

also reduced the fitness of the parasite as reflected in diminished fecundity and tegumental integrity. Given the feasibility of gene editing in both this liver fluke as well as in mammalian cells and model rodent definitive hosts, it should now be feasible to address fundamental questions about this host-parasite relationship and in the associated liver fluke infection-induced bile CCA.

1005

DATA VALIDATION AND REVIEW ACTIVITIES, COMPLEMENTED WITH TRAINING AND MENTORING, IMPROVE DATA QUALITY IN OSUN STATE, NIGERIA

Isaac Adejo[1], Chinedu Chukwu[1], Olabisi Kalejaye[1], Mariah Boyd-Boffa[2], Tom Hall[3], Sonachi Ezeiru[4], Frank Oronsaye[4], Diwe Ekweremadu[4], Perpetua Uhomoibhi[5], Bala Mohammed Audu[5], Ibrahim Maikore[5], Cyril Ademu[5], James Ssekitooleko[6]

[1]*Management Sciences for Health (MSH), Abuja, Nigeria,* [2]*Management Sciences for Health (MSH), Medford, MA, United States,* [3]*Management Sciences for Health (MSH), Arlington, VA, United States,* [4]*Catholic Relief Services (CRS), Abuja, Nigeria,* [5]*National Malaria Elimination Program (NMEP), Abuja, Nigeria,* [6]*The Global Fund, Geneva, Switzerland*

The health management information system (HMIS) is a key component of a strong health system, providing important data to officials at all levels, including those in facilities, to make important decisions and adjustments about health service provision. Management Sciences for Health, in collaboration with the National Malaria Elimination Program and Catholic Relief Services, using resources provided by the Global Fund Malaria Grant (2018-20), works to strengthen the quality of data reported to the national HMIS across seven states. Activities to improve data quality in Osun State include bi-monthly data validation meetings, quarterly data quality assessments, and data review meetings. State data management teams, data validation officers, peer to peer mentoring during validation meetings, and training of Local Government Areas (LGA) staff have supported these efforts. The aim of this study is to identify if this strategy has significantly improved data quality in Osun State, focusing on data correctness. A data validation template, which is an Excel tool that automatically flags data quality issues for seven key malaria performance indicators, was used to identify the rate of data errors generated from the Nigeria HMIS. We compared data before implementation (Jan 2016 to Dec 2017) to data during implementation (Jan 2018 to Dec 2019), looking at the proportion of all health facilities in Osun (819) that had no data errors. The results show that before implementation 28% (230 of 819) of health facilities had no data errors in 2016, and 31% in 2017. During implementation, data quality improved to 62% in 2018 and 90% in 2019 (736 of 819), a 62% improvement compared to 2016. These results indicate that data validation strategies, using data validation officers and peer to peer mentoring during data validation meetings, and training of staff at the LGA level, may contribute to improved data quality. Using the data validation tool enables officials at state and LGA levels to identify errors and provide relevant feedback to health facility staff to make corrections. Improving the tool and its use will continue to be explored.

1006

A COMPARISON OF COVID-19 RESPONSE IN THREE EAST AFRICAN COUNTRIES

Ruth N. Kigozi[1], Simon Peter Kigozi[2], Adoke Yeka[3]

[1]*Malaria Consortium, Kampala, Uganda,* [2]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [3]*Makere University School of Public Health, Kampala, Uganda*

The World Health Organization has declared COVID-19 a pandemic. Three East African community countries reported 591 confirmed cases of COVID-19 with 281 confirmed cases in Kenya, 254 in Tanzania and 56 in Uganda as of 21st April 2020. Each country has implemented the WHO recommended pandemic responses to varying degrees. We examined the correlation between reported number of cases and each country's response. Uganda instituted very stringent measures a few days after reporting of index case including closure of all educational institutions, banning of public gatherings at religious and cultural events, and a ban on public and private transport except for essential services like health services and security. Kenya's response was less stringent with public/religious gathering restricted to less than 15 people, public/private transport allowed but with passenger distancing guidelines and restricting movement in and out of the Nairobi Metropolitan area and three other counties with growing number of COVID-19 cases. By this analysis, Tanzania had not imposed any lock down or social distancing measures since reporting of its index case. A linear regression analysis of new COVID-19 confirmed cases reported per country from the time of reporting index case up to 20th April 2020 (4 - 5 weeks of data) showed that new cases in Kenya increased by 0.28 ($p = 0.007$) cases per day over the study period, while Uganda's new cases decreased by 0.12 ($p = 0.018$) per day. Tanzania's new cases on the other hand increased by 0.51 per day over the study period. These results suggest that, with other factors constant, a more stringent response to COVID-19 was associated with reductions in new COVID-19 infections compared to less stringent approaches.

1007

AN ANALYSIS OF THE IDEATIONAL, BEHAVIORAL AND STRUCTURAL DETERMINANTS OF ANTIMICROBIAL RESISTANCE: A GLOBAL REVIEW OF THE LITERATURE

William Benié[1], Abdul Dosso[1], Thomas Dadie[2], Serge Dali[3], Natalie Tibbels[4], Jeanne Brou[1], Anne Yao[5], Mieko McKay[1], Corinne Fordham[6], Natalie Nguessan[2], Regina Koko[7], Diarra Kamara[1], Danielle Naugle[4]

[1]*Johns Hopkins Center for Communication Programs, Abidjan, Côte D'Ivoire,* [2]*Technical Working Group, Abidjan, Côte D'Ivoire,* [3]*Consultant, Abidjan, Côte D'Ivoire,* [4]*Johns Hopkins Center for Communication Programs, Baltimore, MD, United States,* [5]*National Institute of Public Hygiene, Abidjan, Côte D'Ivoire,* [6]*Johns Hopkins Center for Communication Programs, Baltimore, Côte D'Ivoire,* [7]*US Agency for International Development, Abidjan, Côte D'Ivoire*

Antimicrobial resistance (AMR) occurs when microorganisms such as bacteria, viruses, fungi and parasites evolve in ways that render the medications used to cure the infections they cause ineffective. AMR is primarily propagated by the misuse of antimicrobials among other factors. Breakthrough ACTION supported the national technical working group for AMR in Côte d'Ivoire to conduct a systematic review of the global literature in French and English between 2009 and 2019 on the ideational, behavioral and structural determinants of antimicrobial resistance. The review uncovered 26 relevant journal and grey literature documents. The review organized the behavioral determinants into four groups: incorrect usage of antimicrobials by lay people; incorrect usage by animal and human medical professionals; poor hygiene and sanitation, particularly in medical settings; and the use of antimicrobials in industrial farming and animal husbandry. In the general population, the review found that lack of knowledge of proper use of antimicrobials and potential consequences of misuse and lack of economic resources contributed to misuse of antimicrobials. Among medical professionals, lack of knowledge of proper prescription guidelines, inability to manage patient expectations, desire for autonomy, pressure from peers and pharmaceutical companies, and a lack of understanding of the consequences of misuse of antimicrobials, contributed to misuse. In medical settings, the review found low knowledge of proper hygiene and sanitation practices to be determinants of poor hygiene and sanitation. In industrial settings, lack of economic resources and desire to maximize profits lead to misuse of antimicrobials. Other structural factors contributing to AMR included irregular enforcement around access to antimicrobials, high cost of certain antimicrobials, lack of health insurance, lack of trained animal and human medical professionals, and a lack of laboratories. Breakthrough ACTION and the AMR technical working group in Côte d'Ivoire are developing a national communication strategy to address these factors and slow the progression of AMR.

1008

WHEN WOMEN RETURN TO THEIR NATAL HOMES TO DELIVER: IMPACT ON HEALTHCARE SEEKING AND HEALTH OUTCOMES

Atique Iqbal Chowdhury[1], Asraful Alam[1], Abu Bakkar Siddique[1], Md. Mamunur Rashid[1], Kyu Han Lee[2], Sanwarul Bari[1], Qazi Sadeq-ur Rahman[1], Abu Mohammed Naser[3], Solveig A. Cunningham[3], Shams El Arifeen[1], Emily S. Gurley[2]

[1]*icddr,b, Dhaka, Bangladesh,* [2]*John Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [3]*Department of Global Health, Rollins School of Public Health, Emory University, USA, Atlanta, GA, United States*

In Bangladesh, pregnant women often return to their natal homes to give birth. This temporary migration is motivated by the social support and improved quality of care provided by their extended families. Our objective was to describe the sociodemographic profile of pregnant women who return to their natal home (temporary migrants) and the care they received during and after pregnancy. We used data from a demographic surveillance system established for the Child Health and Mortality Prevention Surveillance (CHAMPS) in Baliakandi, Bangladesh. In 2019, a total of 4,991 women in Baliakandi reported a pregnancy. Of them, 14% returned to their natal home for delivery. Compared to pregnant women who did not go to their natal home for delivery, temporary migrants were younger (mean age 21.3 SD 4.1 vs. mean age 25.4 SD 6.0 p<0.001) and more educated (mean years of schooling 9.3 SD 2.9 vs. mean 7.5 SD 3.2).The average parity (mean 1.4 SD 0.7) of temporary migrants was lower than that of women who remained in Baliakandi (mean 2.4 SD 1.3). Regardless of migration status, the majority of the women reported delivering their babies at health facilities. However, temporary migrants were more likely to give birth at a health facility (79% vs. 61%, p<0.001) and have a skilled birth assistant (84% vs. 66%, p<0.001). Further, temporary migrants were more likely to receive 4 or more ANC visits (40% vs. 29%, p<0.001) and a postnatal medical check-up (66% vs. 47%, p<0.001). Most importantly, temporary migrants had fewer stillbirths (1.3% vs. 2. 6%, p=0.082; OR: 0.7, CI: 0.3-1.4) and neonatal deaths (2% vs. 3.5%, p<0.047; OR: 0.4, CI: 0.2-0.8) compared to women who remained in Baliakandi. In conclusion, pregnant women who visited natal homes to give birth had improved access to care and their children were at reduced risk for poor health outcomes. Health care interventions designed to reduce maternal and neonatal mortality need to account for the environment in the in-laws household in which pregnant women must operate.

1009

A COMPREHENSIVE RADIO-BASED ARCHIPELAGO SYNDROMIC SURVEILLANCE SYSTEM (RASSS) PROVIDED PROSPECTIVE DEFENSE IN ISLANDS COUNTRIES FOR EMERGING RESPIRATORY INFECTIONS

Han-Yi Chiu[1], Hui-Ju Chiang[1], Arata Nathan[2]

[1]*Mackay Memorial Hospital, Taipei, Taiwan,* [2]*Ministry of Health, Majuro, the Republic of the Marshall Islands, Marshall Islands*

Global epidemiology reveals that respiratory viral infections circulate all-year-round in tropical region where the selected virus survived through population transmission might have pandemic potential to countries in other climate zones. This study aims to exam whether the management strategies could promote the reporting rate under radio-based archipelago syndromic surveillance system in the Republic of the Marshall Islands. Further, using this system to evaluate the spreading routes and transmission temporality of the 2016 outbreak of influenza-like illness (ILI) across different islands and atolls. We access to a weekly syndromic surveillance reporting rate and infectious disease cases with ILI were collected from 49 health centers, which managed thousands of islands except for Majuro Atoll and Kwajalein Atoll and under the control of Outer Islands Health Care System (OIHCS) Office. Three Interventions were adopted from June to November 2016. Shipping/flight routes and dates between islands are inquired from Harbor Bureau, Ministry of Transportation and Communications. The reporting rate increased from 62% to 72% after implementing the standard operating procedures (SOP) for reporting and reminding, and the reporting rate even rose to 87% after the intervention of radio announcement and salary deductions for health assistants who didn't complete report. When the outbreak center of ILI occurred in Capital Majuro, there were 5 atolls continuously occurring outbreak. We analyzed the ILI data through RASSS found that the time of ILI outbreak occurred is highly related to the ship's docking time and islands areas but not associated with the distance from Capital Majuro. Accordingly, establishing an efficient surveillance system in resource-limited areas is the most important effort, as we have learned from SARS coronavirus outbreaks in different countries in Asia 2003. Therefore, our study demonstrates that integrating the two arms of medical and management informatics increased the reporting rate so that the epidemiological data can be better used for directing the most appropriate prevention and control measures.

1010

IDENTIFY, DESIGN AND IMPLEMENT CULTURALLY APPROPRIATE STRATEGIES TO APPROACH BANGLADESHI FAMILIES FOR MINIMALLY INVASIVE TISSUE SAMPLING WHEN CHILDREN DIE AT HOME

Shahana Parveen[1], Emily S. Gurley[2], Farzana Islam[1], Sazzad Hossain Khan[1], Tonmoy Sarkar[1], Kamal Ibne Chowdhury[1], Dalia Yeasmin[1], Abdullah Al Masud[1], Muhammad Faruqe Hussain[1], John Blevins[3], Sanwarul Bari[1], Shams El Arifeen[1]

[1]*International Centre for Diarhoeal Diseases Research, Bangladesh (icddr,b), Dhaka, Bangladesh,* [2]*John Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [3]*Rollins School of Public Health, Emory University, Atlanta, GA, United States*

Stillbirths and children aged <5 years who die in health facilities or their home in Bangladesh, are often buried within a few hours, without identifying a definitive cause of death (CoD). Minimally invasive tissue sampling (MITS) can be used to determine CoD but the procedure requires transporting a body from home to a facility, this may not be culturally acceptable, especially after dusk. We explored community members' perceptions and suggestions to identify and design a culturally appropriate strategy to be able to approach families for MITS when children die at home. We conducted 14 key informant interviews and four focus group discussions with families of deceased children, religious and community leaders, and community volunteers in July 2018 and in February 2019 in Baliakandi sub-district, Bangladesh. Our findings guided the design of an initial strategy which we subsequently finalized by piloting through visit to four deceased children's households. The respondents emphasized that community members need to know the details about the MITS procedure before initiation and understand its' link to reducing child deaths. They suggested five steps for approaching a family at home for consent for the MITS procedure:- 1) MITS team to receive the death notification in a short time, within an hour after a child death 2) MITS team, accompanied by a local representative, reach the deceased's household within 30 minutes of notification 3) approach for MITS at daytime and before ritual bathing 4) if consent is given, transport body to the MITS facility from home and return the body within 2-hours - a length of time considered to be tolerable 5) complete ritual bathing at home and burial by the family. After piloting, the final strategy was initiated in June 2019 and by February 2020 the team reached 14 households (of 53 notifications) in daytime along with the local representatives. After approaching for MITS, four consented to participate. The findings suggest that the strategy designed for a sensitive and new method with the involvement of community members is a practical way to reach the grieving family and approach for MITS at home leveraging to identify the COD.

1011

THE HELP MODEL OF STAKEHOLDER ENGAGEMENT FOR GLOBAL HEALTH PROGRAMS

James V. Lavery[1], Emma Z. Richardson[2], Breanna K. Wodnik[1], Michelle Grek[3], Lee Wilkers[3], Susan Landskroener[1]

[1]*Emory University, Atlanta, GA, United States,* [2]*St. Michael's Hospital, Toronto, ON, Canada,* [3]*Emory University, Avondale Estates, GA, United States*

It is common for the stated aims of community and stakeholder engagement (CSE) to include ideas such as empowerment, mobilization, trust-building, education, equity, and acceptance, among many others. But these meta-concepts are vague and complex—socially, politically, economically and ethically—and it is seldom clear precisely what they mean in the context of any given global health research and implementation program (GHP). As a result, there remains a critical gap between the intuitions that lie behind these aspirations and the usual "tools" of evaluation, including "best practices", "lessons", "indicators", "metrics", and "minimum standards", among many others. In this presentation we describe the core logic of the *Human Engagement Learning Platform (HELP) for Global Health* stakeholder (SH) engagement model. The HELP model aims to address one of the core challenges for the planning, design, management and evaluation of CSE in GHP—i.e., that the reasons *why* we pursue CSE remain poorly articulated. The HELP model provides a coherent implementation and evaluation framework organized around four main reasons *why* we use CSE in GHPs: (1) to determine the feasibility and potential value of a GHP for host country SHs; (2) to establish terms of fair cooperation, partnership and accountability between GHPs and their SHs; (3) to help GHPs create anticipated (and unanticipated) value for SHs; (4) to identify and execute specific ethical obligations to SHs. Engagement in relationships with SHs is essential because many of their relevant interests are non-obvious to GHPs and inaccessible through conventional data collection strategies. The HELP model also recognizes that CSE often fails to deliver on the four core aims described above because of a failure to establish the necessary capacities in programmatic and organizational learning. Organizational learning functions as an "operating system" that renders the unique knowledge generation functions of CSE explicit, actionable, and fit for evaluation. We illustrate the HELP model with case examples from our 15 years of empirical and conceptual research on CSE in GHPs in more than 10 countries.

1012

NETWORK MODELS TO IDENTIFY SIGNIFICANT ENTERIC PATHOGEN COINFECTIONS AND THEIR RELATIONSHIP TO ACUTE DIARRHEA IN INFANTS IN DHAKA, BANGLADESH

Connor L. Klopfer[1], Laurent Hébert-Dufresne[1], John P. Hanley[2], Dorothy M. Dickson[2], Marya P. Carmolli[2], Md. Ashraful Alam[3], Benjamin Lee[2], Mami Taniuchi[4], Beth D. Kirkpatrick[2], E. Ross Colgate[2]

[1]*Department of Computer Science, Vermont Complex Systems Center, University of Vermont, Burlington, VT, United States,* [2]*Department of Microbiology and Molecular Genetics, Translational Global Infectious Disease Research Center, University of Vermont Larner College of Medicine, Burlington, VT, United States,* [3]*Maternal and Child Nutrition and Clinical Services Division, icddr,b, Dhaka, Bangladesh,* [4]*Division of Infectious Diseases and International Health, Department of Medicine, University of Virginia, Charlottesville, VA, United States*

Diarrheal disease is the second leading cause of non-birth related death in children under age 5 years in low- and middle-income countries (LMICs). Recent large-scale studies have demonstrated a high prevalence of simultaneous enteropathogen co-infection during infant diarrheal episodes, raising questions about diarrheal etiology. Additionally, studies of acute diarrhea have found co-infection strengthens pathogenesis. Despite these insights, co-occurrence patterns among enteric pathogens and their relative contributions to disease risk remain unexplored. We hypothesize that specific co-pathogen pairings identified by co-occurrence analysis are predictive of whether infant stools are diarrheal versus asymptomatic. To identify patterns of enteric co-infection we used computational approaches to build a bipartite network of participants and enteric pathogens among 791 infants in the MAL-ED and PROVIDE birth cohorts from Dhaka, Bangladesh. Data on enteric pathogen infection in 3,578 diarrheal and 2,951 asymptomatic stools were obtained using a qRT-PCR TaqMan Array Card panel of 40 enteropathogens. For the bipartite network, we randomized the network structure by controlling for the frequency of distinct pathogens per child and the total incidence of each pathogen in the population, then compared the observed data to these null models. Across the network, co-infection with rotavirus and norovirus GII occurred more frequently than expected (157 observed co-occurrences versus expected 95% CI [149.7, 154.8]), as did ETEC and adenovirus co-infection (349 observed, 95% CI [320.9, 339.0]). Importantly, 75% of stools with co-occurring rotavirus and norovirus GII were diarrheal, as opposed to asymptomatic, and 91% of stools with ETEC and adenovirus co-infection were diarrheal. As we move beyond the One Pathogen, One Response paradigm, interventions to prevent diarrheal disease in infants in LMICs must account for the presence of co-pathogen infections. Identifying significant co-infection patterns will inform treatment approaches and vaccine development to eliminate the excess burden of diarrheal disease.

1013

TOWARDS UNIVERSAL HEALTH CARE: ECONOMIC COSTS AND BENEFITS OF COMMUNITY HEALTH WORK IN RWANDA

Janna M. Schurer[1], Kelly Fowler[2], Ellen Rafferty[3], Ornella Masimbi[1], Olivia Rozanski[2], Hellen Amuguni[2]

[1]*University of Global Health Equity, Kigali, Rwanda,* [2]*Cumming School of Veterinary Medicine at Tufts University, North Grafton, MA, United States,* [3]*University of Alberta, Edmonton, AB, Canada*

In Rwanda, where the number of health workers is well below the World Health Organization threshold for critical shortage, Community Health Workers (CHWs) are instrumental in linking rural populations to health services. CHWs live in the communities that they serve and are trained to deliver services focused on controlling infectious diseases, promoting health literacy, and improving maternal/child health. CHWs are elected by their communities and do not receive annual salaries for their services. The CHW program in Rwanda is widely lauded as contributing to unprecedented improvements in population health; yet, little is known about the social, economic, and emotional situation of this group. This study aimed to (1) assess CHW opportunity costs, (2) describe CHW motivation and job satisfaction, and (3) solicit CHW ideas for increasing retention and delivery of quality services. Therefore, we conducted a mixed-method evaluation, performing in-depth interviews with 145 CHWs from 3 districts to obtain data on household economics and perspectives on care delivery. The results indicated that each CHW volunteered an average 4 hours per day throughout the year. They lost a median 127 684 RWF per year (range: 2 359 - 2 247 807 RWF/yr) in opportunity costs and spent an additional 36 228 RWF per year (range: 3 600 - 364 800 RWF/yr) in out-of-pocket expenses related to their CHW duties. CHWs identified many positive reasons for volunteering. These focused on social benefits, healthcare training, and gratification in helping others. Participants also identified challenges, including supply shortages, transportation, long hours, lack of formal workspaces, and lack of salary. Their ideas for reasonable incentives to improve retention and patient services included transport support (e.g. bicycles), more frequent training, waivers for school fees or community-based health insurance, and formal workspaces. Overall, this suggests that task shifting from professional health workers to CHWs could continue to be a successful strategy for addressing worker shortages but that increased resources are needed to ensure that CHWs are adequately provisioned.

1014

STRENGTHENING MALARIA DATA CAPTURE AND ADDRESSING DATA ACCURACY THROUGH TARGETED SUPPORTIVE SUPERVISION OF HEALTHCARE WORKERS AT FACILITY LEVEL: EARLY LESSONS IN PROGRAMMING

Hellen Gatakaa[1], James Andati[2], Christine Mbuli[3], Willis Akhwale[2], Gladys Tetteh[4], Lolade Oseni[4]

[1]*Jhpiego, Nairobi, Kenya,* [2]*USAID PMI – Impact Malaria Project, Nairobi, Kenya,* [3]*Division National Malaria Program, Ministry of Health, Nairobi, Kenya,* [4]*Jhpiego, Baltimore, MD, United States*

Health facility data reported through the Kenya Health Information System (KHIS) form the primary data for malaria programmatic monitoring. The Kenya Malaria Program Review in 2018 determined that while completeness and timeliness of reporting per KHIS was above 95%, not all malaria cases during the period 2013-2017 were reported into the KHIS due to suboptimal data aggregation. Data quality audits (DQAs) conducted in 2017 showed discrepancies between values in primary registers and summary tools, compromising data fed into KHIS. To implement some of the corrective actions recommended by annual DQAs, Kenya's National Malaria Program, with support from the PMI Impact Malaria (IM) project, integrated a data validation and correction exercise into the quarterly Supportive Supervision (SS) process. The SS tool was revised to include validation of data in primary registers, to monitor and improve accuracy of malaria data in both the primary registers and summary tools. From October 2019 to January 2020, targeted SS was conducted in 28 of 186 facilities identified to have poor data at project baseline (April 2019). The SS teams identified that 53% of confirmed malaria cases were under-reported while cases treated with artemether-lumefantrine were correctly reported, thereby creating a picture of over-treatment in KHIS reports. Immediate coaching was done to correct health worker knowledge and competence in data capture and aggregation, followed by onsite correction of data aggregation errors. After the first round of SS, a review of SS data showed an increase in the percentage of health workers able to correctly capture and aggregate data and a review of KHIS data showed a decrease in facilities that reported treating more than the confirmed cases (69% in 2019 versus 75% in 2018). The relative decrease in number of facilities incorrectly reporting over-treatment was higher (16%) in IM intervention facilities compared to non-intervention facilities (4%). With integration of data validation and correction into continuous SS rounds, IM expects further improvements in data capture and aggregation and more accurate reporting of malaria cases into the KHIS.

1015

BUILDING CAPACITY AND INFRASTRUCTURE AT HOSPITALS IMPLEMENTING MINIMALLY INVASIVE TISSUE SAMPLING

Anna Marie Aceituno[1], Gervais Ntakirutimana[2], Marie Claire Ndayisaba[2], Djibril Mbarushimana[3], Elisé Hategekimana[3], Nuwadatta Subedi[4], Christina Paganelli[1], Norman Goco[1]

[1]*RTI International, Research Triangle Park, NC, United States,* [2]*Kigali University Teaching Hospital, Kigali, Rwanda,* [3]*Butare University Teaching Hospital, Butare, Rwanda,* [4]*Gandaki Medical College, Pokhara, Nepal*

The Minimally Invasive Tissue Sampling (MITS) Surveillance Alliance is a global, multidisciplinary consortium funded by the Bill & Melinda Gates Foundation, which aims to expand the use of MITS globally and improve the quality of global mortality data. The MITS Alliance has catalyzed the expansion process with the development and administration of a MITS grant program. The program provides technical assistance and facilitates the implementation of small-scale mortality surveillance studies. To date, 17 organizations have been awarded MITS grants. Most MITS grantees are in countries where complete diagnostic autopsy is neither routine nor culturally acceptable and post-mortem examination is not incorporated into clinical practice. Prior to receiving MITS grants, some hospitals' mortuaries were not functional. We present case studies of three MITS grantees at the University Teaching Hospital of Butare (CHUB), Rwanda, the University Teaching Hospital of Kigali (CHUK), Rwanda, and the Gandaki Medical College of Pokhara, Nepal, on how they have used the grants to build infrastructure and capacity for post-mortem investigation. Strategies included 1. Renovating and expanding mortuaries to facilitate research and increase capacity; 2. Improving infection control infrastructure, such as installing hand-washing stations; 3. Training additional mortuary staff in infection control and proper use of personal protective equipment; 4. Educating clinical staff about the value postmortem studies add to more accurately determine cause of death; 5. Hiring psychosocial staff to provide support to families of the deceased; 6. Emphasizing how more precise cause of death data can ultimately improve clinical care within the hospitals; and 7. Working closely with hospital administration to ensure services will be sustained after the grant period. Considering the SARS-CoV-2 pandemic, these case studies will also highlight how the improved hospital infrastructure and training affected their ability to respond to SARS-CoV-2-related mortality within the three hospitals, as well as post-mortem infection control.

1016

PATTERNS OF MOBILITY AND ITS IMPACT ON RETENTION IN CARE AMONG PEOPLE LIVING WITH HIV IN THE MANHIÇA DISTRICT, MOZAMBIQUE

Edson L. Bernardo[1], Tacilta Nhampossa[1], Denise Naniche[1], Troy D. Moon[2], Kate Clouse[2], James G. Carlucci[2], Moshin Sidat[3]

[1]*Health Research Center of Manhica, Maputo, Mozambique,* [2]*Vanderbilt Institute for Global Health, Vanderbilt, TN, United States,* [3]*Eduardo Mondlane University, Maputo, Mozambique*

Retention in HIV care and treatment has been a significant challenge in Mozambique since the inception of its HIV programs in the early 2000´s. Significant efforts have been made to address the epidemic in Mozambique, yet as of the March 2019 only 49% of its HIV-infected population was receiving ART and only 77% of those on treatment had undetectable viral loads. One potential challenge to high retention in HIV care and treatment that is gaining attention is the mobility of people between regions and countries. Therefore, with this study we sought to describe the patterns of mobility among PLHIV seeking care at the Manhiça District Hospital (MDH) in southern Mozambique. Results may be of importance to Mozambique and other countries in the region with large migratory populations as they strive to improve effectiveness of HIV care and treatment services. The study took place at the Health Research Center of Manhiça (*Centro de Investigação de Saúde de Manhiça*, CISM) at MDH of Manhiça District, Mozambique. A cross-sectional survey was conducted to collect demographic information and mobility history. We then linked survey data to participant clinical and demographic data from the HIV care and treatment program. A total of 390 HIV-positive adults on ART were included in this study. Among mobile study participants, 68% reported leaving the district 2-5 times over the 12-month period prior to survey administration, with 38% reporting they were away from Manhiça 15 days to 3 months each trip and another 25% reporting they were away 3-9 months each trip. Overall, only 68% of our mobile population reported that they had access to their ART medications when traveling. Clinical data based on pharmacy pick-up dates for both our mobile and non-mobile participants, showed that 30% of our total population had at least one delay (15-60 days late) in ART pick-up for the 12-month period prior to survey administration, and 11% had at least one documented delay in ART pick-up of >60 days in ART, thereby meeting the lost-to-follow-up designation per national protocols.

1017

IMPROVING THE QUALITY OF MALARIA SERVICE DELIVERY BY COMMUNITY HEALTH OFFICERS THROUGH INTERNSHIP TRAINING IN GHANA

James Sarkodie[1], Akosua Gyasi[2], Amos Asiedu[3], Eric LaFary[3], Richard Dogoli[3], Raphael Ntumy[3], Keziah Malm[2], Lolade Oseni[4], Gladys Tetteh[4]

[1]*Impact Malaria Ghan, Accra, Ghana,* [2]*National Malaria Control Programme, Accra, Ghana,* [3]*Impact Malaria Ghana, Accra, Ghana,* [4]*Jhpiego, Baltimore, MD, United States*

Ghana implements Community-based Health Planning and Services (CHPS) as a strategy for achieving universal health coverage of essential primary health services at the community level. While Community Health Officers (CHOs) who provide these essential services at CHPS sites receive formal pre-service training, they tend to lack adequate knowledge and skills to provide quality malaria service delivery. In 2019, the Impact Malaria project supported the National Malaria Control Program (NMCP) to implement "CHO internship training" to improve CHO skills and address these challenges. Sixty CHOs selected from 60 CHPS sites in 12 districts were trained over five days by district clinicians. Material was based on NMCP malaria guidelines. Following training, CHOs received in-service mentoring over two months by the same clinicians through remote coaching and supportive supervision visits. The level of reported malaria testing, adherence to negative test results and administration of intermittent preventive treatment (IPTp) were selected as performance indicators. Pre- and post-training assessments revealed immediate improvements in malaria knowledge from 37% to 70% and patient assessment skills from 25% to 74%. All 60 CHOs received post-internship follow-up visits two months after the internship training. Follow-up assessments revealed that there was no decline in skills. Four months after the internship, malaria testing rates for the 60 CHPS sites improved from 96% to 99.8%, and suspected uncomplicated malaria cases tested negative but treated as malaria reduced from 0.3% to 0%. IPTp1 and IPTp3 coverage increased from 59% to 64% and 36% to 41% respectively. The visit also revealed ongoing mentorship between the trainers and the CHOs, improvement in referral efficiency and improved availability of health commodities. CHO internship training is an effective approach to build the capacity of CHOs for quality malaria service delivery and the model of training is replicable in other technical areas.

1018

HUMAN AND COMPUTER-GENERATED REMOTE ENUMERATIONS OF SATELLITE IMAGERY: A COMPARATIVE ANALYSIS AGAINST A FIELD VERIFIED GOLD STANDARD

Anne Martin[1], Frazer Bwalya[1], Derek Pollard[1], Hugh Sturrock[2], Claire Dooley[3], Heather Chamberlain[3], Olena Borkovska[4], Rhiannan Price[5], Silvia Renn[6], Hannah Koenker[7], Emmanuel Kooma[8], Anna Winters[1], John Miller[9]

[1]*Akros, Lusaka, Zambia,* [2]*University of California San Francisco, San Francisco, CA, United States,* [3]*WorldPop, University of Southampton, Southampton, United Kingdom,* [4]*Center for International Earth Science Information Network, New York, NY, United States,* [5]*Maxar Technologies, Denver, CO, United States,* [6]*Afgeo, Lusaka, Zambia,* [7]*Johns Hopkins Center for Communication Programs, Baltimore, MD, United States,* [8]*National Malaria Elimination Centre, Ministry of Health, Lusaka, Zambia,* [9]*PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Lusaka, Zambia*

Enumeration of residential structures from satellite imagery has emerged as a useful process for estimating population and resource needs in public health, particularly in settings where census and headcount estimates of population differ or may miss large swaths of rural locations. As satellite imagery has become more widely accessible and used, the next step for innovation and efficiency of enumeration lies in automation of these processes via artificial intelligence (AI). We have yet to produce thorough validation of these AI-driven processes in a public health context. In this analysis, we will compare a multiyear, sub-national, dataset of over 700,000 observations with datasets generated using feature extraction from satellite imagery and associated derivatives. We will assess relative accuracy, identify ideal strategies for enumeration processes, and improve the algorithms that create these datasets. The following datasets are included: 1. AI-predicted building footprint layer, 2. AI-predicted building footprint layer with algorithm-derived structure attributes, 3. settlement layer built off of AI-predicted footprint layer, 4. remote human-enumerated building footprint layer, 5. 100m gridded population estimates raster and 6. field-verified structure classification and household size data, which, despite a few limitations, will serve as the gold standard. We will compare each dataset to the gold standard, reviewing variables that evaluate building footprint, classification, settlement existence, and population. The results will present the comparisons of these variables and trends with respect to their accuracy, in statistical models where possible. The aim of this analysis is to establish a decision matrix to guide country governments and stakeholders on how to incorporate spatial datasets into planning of health campaigns: for example, in the procurement of commodities and micro-planning of human resources for insecticide treated net mass distribution or mass immunization. We will interpret the results in the context of the tools and limitations at the disposal of health ministries and health centers.

1019

PROTECTING PEOPLE IN LONG-TERM CARE FACILITIES FROM COVID-19 BY ROUTINE TESTING OF EMPLOYEES: A MODELING APPROACH

Nessma Adil Mahmoud Yousif[1], Looli Alawam[2], Pierre Ngougoue Ngougoue[3], Henri Christian Tsoungui Obama[3], Gideon Ngwa[4], Martin Eichner[2], Kristan A. Schneider[3]

[1]*AIMS Cameroon, Limbe, Cameroon,* [2]*University of Tubingen, Tubingen, Germany,* [3]*University of applied sciences of Mittweida, Mittweida, Germany,* [4]*University of Buea, Buea, Cameroon*

The COVID-19 pandemic with its high infectiousness is a public health emergency of international concern, particularly threatening the lives of senior citizens, that forced policymakers to implement draconic control interventions affecting the global economy and restricting civil rights. With borders closed many countries face the problem of efficiently reacting to the pandemic due to inaccurate case detection and a potentially high number of unreported cases. With data being initially sparse and heavily biased, health management decisions need to be guided by model predictions. The CDC identified people living in long-term care facilities (LTCF) as a high-risk group. The protection of such citizens against the COVID-19 is a priority in disease management. This requires regular testing of staff working in LTCFs. With a limited capacity of reliable PCR tests, monitoring LTCFs needs to be carefully optimized. We present a predictive model, that is an extension of the pandemic preparedness tool CovidSIM Version 1.1 (http://covidsim.eu/) that considers the interactions between three groups: the general population, the risk group living in LTCFs, and the LTCF staff. We study the effect of routine testing and isolation of LTCF staff as a measure to protect the risk group. The model helps to optimize the testing schemes of LTCF staff to efficiently protect the risk group. The model allows contrasting the economic gain of protecting the risk groups against the costs for regular COVID-19 testing. We present simulation results obtained by using the software Berkeley Madonna roughly reflecting the situation in the Federal Republic of Germany.

1020

ASSESSING THE ETHICAL COMPLEXITIES OF CONFIDENTIALITY DURING APPROACH AND FOLLOW-UP ON MORTALITY SURVEILLANCE

John Blevins[1], Maria Maixenchs[2], Ahoua Kone[1], Emily S. Gurley[3], Faruqe Hussain[4], Shahana Parveen[4], Berhanu Damise[5], Ketema Degefa[5], Peter Otieno[6], Kounandji Diarra[7], Tieman Diarra[7], Rui A. Guilaze[8], Nellie Myburgh[9], Baindu Kosia[10], Khatia Munguambe[11]

[1]*Emory University Rollins School of Public Health, Atlanta, GA, United States,* [2]*ISGlobal, Barcelona, Spain,* [3]*Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, United States,* [4]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh,* [5]*Haramaya University, Harar, Ethiopia,* [6]*Kenya Medical Research Institute, Kisumu, Kenya,* [7]*Centre for Vaccine Development, Mali, Bamako, Mali,* [8]*Centro de Investigação em Saúde de Manhiça, Manhica, Mozambique,* [9]*University of the Witwatersrand, Johannesburg, South Africa,* [10]*FOCUS1000, Makeni, Sierra Leone,* [11]*Eduardo Mondlane University, Maputo, Mozambique*

The Child Health and Mortality Prevention Surveillance (CHAMPS) program conducts minimally invasive tissue sampling (MITS) to identify the causes of <5 child deaths in 7 sites in countries in Africa and Asia. Families are approached in a health facility or in their homes soon after the death of a child for potential inclusion in the program because MITS must be conducted <24 hours after death. Because some families are approached when neighbors and extended family are present to offer support, special consideration must be taken to maintain families' confidentiality as they deliberate on their participation. Similar considerations arise again when families receive findings on the cause of death because those extended same social networks know that such follow-up will occur. CHAMPS sites conducted qualitative studies to identify the most important challenges to confidentiality. Through semi-structured interview, observation, and focus group discussions, teams gathered input from families and key stakeholders on the best approaches. All sites identified the complexity of approaching the family when so many community members were present at the family home. 6 of 7 sites identified challenges that arise when grandparents expect to be involved at the initial approach for consent (because the sites identify the parents as those from who consent should be obtained) and during the meetings to present cause of death findings (because of confidentiality concerns). Six sites also identified a similar challenge in relation to neighbors and extended family. The research demonstrates the influence of cultural practices and norms that must be considered alongside ethical principles of autonomy, nonmaleficence, beneficence, and fidelity. Considering these cultural factors alongside these ethical principles led teams to develop more comprehensive procedures for family approach and follow-up (for example, in developing family-specific plans for follow-up processes and location) that went beyond procedures stipulated in approved protocols in order to reflect parents' priorities and values while maximizing the ethical principles involved.

1021

CLINEPIDB.ORG: GLOBAL HEALTH DATA SHARING, SEMANTIC HARMONIZATION AND EXPLORATORY DATA ANALYSIS

Brianna Lindsay[1], Cristina Aurrecoechea[2], John Brestelli[1], Brian Brunk[1], Danielle Callan[1], Dave Falke[2], Steve Fischer[1], Danica Helb[1], Jay Humphrey[2], John Judkins[1], Jessica C. Kissinger[2], David S. Roos[1], Sheena Shah Tomko[1], Christian J. Stoeckert Jr[1], Jie Zheng[1]

[1]*University of Pennsylvania, Philadelphia, PA, United States,* [2]*University of Georgia, Athens, GA, United States*

ClinEpiDB (https://clinepidb.org) is an open-access online resource that enables investigators to maximize the utility and reach of research and make optimal use of data released by others. Standardization of research-oriented epidemiologic data collection is generally not routine. A component of ClinEpiDB data integration includes mapping data variables to OBO Foundry ontology terms. To investigate consistency across datasets we evaluated how many terms were common across all datasets. Studies included in ClinEpiDB thus far come from three major domains: maternal, newborn & child health; malaria; and neglected tropical disease, largely dictated by priorities of ClinEpiDB funding. As of April 2020, ClinEpiDB contains data from 30 global health studies. This includes 5 case-control studies, 9 longitudinal cohort studies, 8 cross-sectional surveys and 8 randomized controlled trials. From an analysis of over 5,000 variables in 30 datasets we found that the most commonly used variable categories were: demographics (30/30 datasets; 100%), laboratory test results (27/30; 90%), signs and symptoms (23/30; 77%), physical examination (20/30; 67%) and anthropometrics (18/30; 60%). The most common variables across datasets include: sex (30/30; 100%), participant ID (27/30; 90%), weight (18/30; 60%), age at enrollment (17/30; 57%), temperature (16/30; 53%), height (14/30; 47%), and household assets (~12/30; 40%). Efforts by the WHO and other global health governing bodies rely on standardization of global health indicators and this analysis suggests significant overlap already exists in constituent data variables and data types of interest. Making standardized tools available further upstream in study conceptualization and protocol design stands to gain efficiencies downstream in data sharing, reporting and clinical translation of results.

1022

ENHANCING COMPETENCE OF HEALTH FACILITY WORKERS TO USE DATA TO IMPROVE INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY (IPTP) UPTAKE, LIBERIA

Lauretta Nagbe[1], Gaspar Mbita[1], Birhanu Getahun[1], Jessica Kafuko[2], Joseph O. Alade[3], Maweyata Sow[1], Gladys Tetteh[4], Lolade Oseni[4], Shelly Wright[5], Anne Fiedler[1]

[1]*USAID Maternal and Child Survival Program/Expansion of Malaria Services (MCSP/EMS, Jhpiego Liberia, Monrovia, Liberia,* [2]*U.S. President's Malaria Initiative (PMI), U.S. Agency for International Development (USAID), Monrovia, Liberia,* [3]*National Malaria Control Program (NMCP) Ministry of Health (MOH), Monrovia, Liberia,* [4]*Jhpiego Johns Hopkins University, Baltimore, MD, United States,* [5]*U.S. Agency for International Development (USAID), Monrovia, Liberia*

In Liberia, health facilities (HFs) compile and send summary health information data to the district or county level to enter into the DHIS2 monthly. Lack of computers and the internet at many HFs hinders their access to the DHIS2, thereby limiting data analysis and visualization to inform managers and health care workers (HW) to take actions that could improve service quality and uptake at HF level. The Liberia 2016 Malaria Indicator Survey showed that only 22% of pregnant women received three IPTp doses. HW competence and leadership to analyze, visualize and use data is critical to improve services delivery. In May 2019, to address some of the low IPTp3 uptake challenges, the USAID MCSP/EMS in collaboration with the NMCP/MOH and other partners developed and implemented a facility-based malaria performance indicator tracking tool called Wallchart at 22 HFs. We conducted one-day on-site training and mentorship for Antenatal Care (ANC) staff on ANC data recording, aggregation reporting, plotting IPTp3 uptake data on the wallcharts and data interpretation. Seven months later, we analyzed DHIS2 data to assess IPTp3 uptake, and a self-administered questionnaire to assess HWs' competence in using and interpreting the Wallcharts. Two-population z-test was used to measure the changes of IPTp3 uptake. Over 80% of HWs expressed that the Wallcharts were user-friendly and above 90% were able to plot correctly IPTp3 data in the Wallchart and interpreted it. Managerial actions implemented based on the Wallcharts use include: adequately quantifying Sulfadoxine-Pyrimethamine (SP) with timely requisition at service delivery points, reduced SP stockout, and provision of health education for pregnant women about benefits of at least three doses of IPTp. The proportion (PR) of IPTp3 uptake increased by 17% from 32% in May 2019 to 46% in November 2019 (PR=17%; 95%CI: 5-29%; p<0.0073; z=2.6817). The use of Wallcharts informed HF staff and managers to monitor IPTp3 uptake and took corrective measures which led to a substantial increase of IPTp3 uptake. NMCP and its partners will roll-out the use of Wallcharts in 12 of the 15 counties in Liberia.

1023

PERFORMANCE OF THE GUARAL+ST MOBILE APP IN ASSESSING THERAPEUTIC RESPONSE IN CUTANEOUS LEISHMANIASIS PATIENTS IN COLOMBIA

Alejandra Maria Del Castillo[1], Maria Del Mar Castro[1], Alexandra Cossio[1], Ruth Mabel Castillo[1], Domiciano Rincon[2], Andres Navarro[2], Neal Alexander[1]

[1]*Centro Internacional de Entrenamiento e Investigaciones Medicas-CIDEIM, Cali, Colombia,* [2]*Universidad Icesi, Cali, Colombia*

Leishmaniasis is a global health problem. Data regarding effectiveness of antileishmanials is limited due to geographical and economic barriers. The use of mHealth may contribute to improve access to therapeutic monitoring. Recently, a mobile app (Guaral+ST) was designed to remotely determine the therapeutic response in patients with cutaneous leishmaniasis (CL) through photographs taken by community health volunteers (CHV). In this study, we aim: 1) Estimate the reproducibility of the evaluation of the therapeutic response using photographs of lesions of CL patients, taken with the Guaral+ST app; and 2) Determine the validity of the Guaral+ST app to remotely assess the therapeutic response of CL patients. Patients of any age receiving antileishmanial treatment were eligible. Trained Community Health Volunteers (CHV) used Guaral+ST app to make photographic monitoring of lesions at 13- and 26 weeks post-treatment. Photos taken by CHV were evaluated by 3 physicians who independently defined the therapeutic response. The result of these observers was compared to the therapeutic response defined by an expert physician who directly assessed patients' lesions (reference standard). Seventy-two participants were included. To date, 24 had completed follow-up. Patients were mostly men 15/24 (62.5%), afro-descendants (70.8%), with mean age of 27 (SD:13.3) years. Median duration of disease was 2 months (range: 0.5-9). In total, 154 images were evaluated, of which 15 (10.6%) were non-interpretable due to low quality (14/15 were out of focus). Kappa index between observers and the reference standard was 0.72 (95% CI: 0.72-1.00). Kappa between observers=0.63 (95% CI: 0.34-0.84). Sensitivity of Guaral+ST was 100% (95% CI: 54.1-100), specificity=100% (95% CI: 47.8-100), positive predictive value=100% (95% CI: 54.1-100), negative predictive value =100% (95% CI: 47.8 -100). These preliminary results support the remote assessment of the therapeutic response in CL patients using Guaral+ST app. This could be an alternative to improve access to therapeutic follow-up of LC in remote areas where follow-ups can be carried out by trained CHV.

1024

ADDRESSING THE MENTAL HEALTH OF PERSONS LIVING WITH LYMPHATIC FILARIASIS IN LÉOGÂNE, HAITI: EFFECTIVENESS OF A CHRONIC DISEASE SELF-MANAGEMENT PROGRAM

Sarah Bazur-Leidy, Luccene Desir, Martha Desir, Lauren Paul, Cassandra Bryan, Tsion Horra, Samhita Kumar, Gregory Noland, Eve Byrd

The Carter Center, Atlanta, GA, United States

There is mounting evidence that neglected tropical diseases (NTDs) are major drivers of common mental health disorders and psychosocial morbidity and a growing recognition of the role of mental well-being in NTD prevention and morbidity management. Yet there are few validated interventions for addressing the burden of chronic NTDs and co-morbid mental disorders, especially in contexts affected by large-scale poverty, insecurity, and fragility. This study aims to fill this gap by evaluating the effectiveness of a chronic disease self-management (CDSM) intervention to improve mental health outcomes among persons with lymphatic filariasis in Leogane, Haiti. CDSM is a six-session intervention delivered by animatrices, or peer facilitators. The study employs a randomized, waitlist-controlled design implemented among clusters of LF patient support groups ("Hope Clubs"). Mental health outcomes were scored across five domains using contextualized tools where available: chronic disease self-efficacy, social support, depression, self-rated health, and disability. Baseline data revealed high prevalence of depressive illness symptoms among both the intervention group (n=118, 48%) and control group (n=92, 52%). There was no significant difference in depressive illness or demographic factors between the two groups. Following CDSM implementation in Arm 1, change within each group and between groups was assessed using t-tests. Analysis of the between-group change revealed higher self-efficacy in chronic disease management in the intervention arm compared to the control arm (p=.04). Between-group analysis further showed no significant change between arms in the domains of depression (p=.51) disability (p=.51), self-rated health (p=.65), or social support (p=.78). However, a 25.9% reduction in prevalence of depressive symptoms was noted in Arm 1; similarly, a 33% reduction was observed in the control arm. Amid growing demand for mental health and psychosocial support services for persons living with NTDs, such an intervention that can be delivered by peer facilitators and integrated into present systems of care is highly attractive.

1025

UTILIZATION OF A HEALTH-RELATED DATA COLLECTION TOOL DURING SHORT-TERM EDUCATIONAL MEDICAL TRIPS TO DEVELOPING COUNTRIES FOR SURVEILLANCE AND REPORTING

Etienne Jaime[1], Joe Bryan[1], Julissa Grullon[2], Dulfelina Cruz[3], Mayelin Peña[2], Alexandra Amador[3], Xiomara Erazo[4], Zaira Alvarado[5], Pedro Solano[5], Harold R. Garner[6], Cameron Sumpter[6], Dean Sutphin[1]

[1]*Edward Via College of Osteopathic Medicine, Blacksburg, VA, United States,* [2]*Oscar de la Renta Pediatric Center, Veron, Dominican Republic,* [3]*Veron Rural Clinic, Veron, Dominican Republic,* [4]*James Moody Adams Clinic, Tegucigalpa, Honduras,* [5]*Shalom Family Medical Center, Santiago Texacuangos, El Salvador,* [6]*Edward Via College of Osteopathic Medicine, Spartanburg, SC, United States*

The Edward Via College of Osteopathic Medicine (VCOM) provides osteopathic students an opportunity to participate in international medical outreach trips in their second year. VCOM has a north-south collaboration with partners in the Dominican Republic, El Salvador and Honduras for care of underserved populations. Since 2017, a VCOM developed electronic data collection tool, Clinical Rotation Evaluation and Documentation Organizer (CREDO), has been used to record patient care using ICD-10 Codes. The objective is to describe clinical findings of the patients attended during the medical outreach trips by country, age, gender and season in order to tailor medical services. Since 2017, 900 students and their preceptors have attended 7652 patients (3595, Dominican Republic; 2075, El Salvador; 1982, Honduras) including 2696 (35%) male and 4956 (65%) female. Children 0-14 years constitute 43% (n=3340); adults 19-44 years 27% (n=2096); 45- 64 years, 15% (n=1177); ≥65 years, 7.5% (n=571). Overall, the most common diagnoses (dx) are Infections and Parasitic Diseases (Inf) (18%), Diseases of the Respiratory System (Resp) (17.7%), Diseases of the Digestive System (Dig) and Diseases of the Musculoskeletal System and Connective Tissue (Musc) (7.9% each). There were only slight differences in ranking by country. By gender, Diseases of the Nervous System (headache, etc.) were more prevalent in females (7.1% vs 3.8%) as were Genitourinary diagnosis (5.6% v 2.1%). Among children 0-14 the most common diagnoses were Inf and Resp accounting for 56.4% of the diagnosis (28% each). Skin and soft tissue dx (5.5%) were twice any other ages. In adults, Musc complaints were prominent (13.4%) and increased with age reaching 18% in those ≥65 years. Some variations between the wet (July and Oct.) and dry seasons (January and April) were observed. Overall, and consistent among all sites, Inf and Resp dx were higher during the dry season (19.1 and 19.5%) compared to the wet season (16.8% and 15.9%). Dig dx were higher in the wet season (8.3%) compared with the dry (7.4%). These findings provide the basis for tailored services to each participating region, gender, age and season.

1026

EXPANDED PRIMARY CARE IN BUGESERA DISTRICT, RWANDA: OPPORTUNITIES FOR INCREASING PREVENTIVE SERVICES

Donald S. Shepard[1], Regis Hitimana[2], Maria Kulchyckyj[1], Sabine F. Musange[2]

[1]*Brandeis University, Waltham, MA, United States,* [2]*University of Rwanda School of Public Health, Kigali, Rwanda*

The Rwanda Ministry of Health selected Bugesera district, 42 km south of Kigali, Rwanda's capital, for expanding primary health care. In 2019, the MoH opened 8 "Second Generation of Health Posts" in Bugesera, within walking distance of many potential patients. These facilities, equipped with laboratories, were especially targeted at three populations: pregnant women, children under 5, and adults age 50 and above (for controlling chronic diseases). Existing data systems, such as Rwanda's Health Management Information System (HMIS), miss health needs outside of health facilities. To obtain needed data, we conducted focus group discussions and household cluster surveys of 320 randomly selected residents from each of these target populations. These data, collected just prior to the posts' opening, uncovered key strengths and weaknesses of the existing health system. Among pregnant women, while coverage of antenatal testing for HIV/AIDS was almost perfect (99.7%), testing for syphilis was only fair (49.3%). Thanks to Rwanda's high coverage of community-based health insurance (*mutuelles*), 91.8% of pregnant women and 79.0% of adults reported having insurance for their recent services, mostly received at health centers. While this high coverage meant that monetary costs were generally affordable, patient visits required substantial time investments. A first antenatal visit averaged 6.30 hours on site and the latest child health visit averaged 4.26 hours, including travel time. Adults reported receiving few preventive services within the past year. Only 29.0% of adult respondents had received a blood pressure check and only 24.5% a urine check (which could screen for diabetes). Only 32.3% had been asked about smoking and only 34.4% about alcohol use. In conclusion, these new health posts have the potential to build on Rwanda's solid financial base for primary care and raise the coverage of all preventive services to the same high level as HIV/AIDS testing. Global experience suggests Rwanda can achieve this through protocols and staff training to emphasize preventive services and maximize patients' knowledge, comfort, and satisfaction.

1027

DRIVING FORCES BEHIND TIMING AND DECISION-MAKING FOR U.S. PUBLIC AND PRIVATE UNIVERSITIES DURING THE COVID-19 PANDEMIC

Michael E. von Fricken, Kevin Cevasco, Hayley North, Rachel Wofford, Sheryne Zeitoun, Abigail Gregory, Maha Hassan, Graham Matulis, Aya Abdo, David Farris, Amira Roess

George Mason University, Fairfax, VA, United States

The COVID-19 pandemic has resulted in an unprecedented disruption of higher education, requiring public and private universities across the United States to cancel travel, shift to online learning, empty campus housing, and close campus for all non-essential personnel. This project compiles critical details regarding key actions taken during the initial response to COVID-19 and compares them with state prevalence, governor political affiliation, state of emergency announcements, and if the program has a school of medicine or public health. An in-depth original database of COVID-19-related policy changes across 591 U.S. public and private universities, which includes all 50 states and impacts over 8 million faculty, staff, and students was compiled. Data was gathered through university COVID-19 campus updates and announcements, covering announcements from Feb 26th to March 31st. All data have been cleaned to examine variables potentially contributing to differences in university decision making at the state and national level, and differences between private and public university announcement timelines. Full analysis pending, but preliminary observations suggest clear differences between the timing of private and public university decision making as well as variations observed between states and within states. The swift, severe, and global nature of COVID-19 offers a unique opportunity to expand knowledge and provide insights about risk communication, decision-making, and response at the university level during a country-wide public health pandemic emergency. The importance of coordinating university decisions in an emergency scenario cannot be understated, with stark variations observed in decision and announcement dates, leading to mixed messaging and possibly reducing effectiveness of early interventions.

1029

EMPOWERMENT THROUGH EDUCATION: A QUALITATIVE ANALYSIS OF THE SOCIO-CULTURAL AND ECONOMIC FACTORS IMPACTING MAASAI GIRLS' EDUCATIONAL ATTAINMENT IN LAIKIPIA COUNTY, KENYA

Margaret E. Crampton, Saumya Kumar

Frank H. Netter School of Medicine at Quinnipiac University, Hamden, CT, United States

Education is an effective way to improve girls' self-worth, future success and health. Curtailing a girl's education creates substantial negative consequences, at both individual and societal levels. In Laikipia County, Kenya roughly 60% of girls are estimated to have dropped out of school by the secondary school level. This formative research targeted the IL Ngwesi Ranch, a region in Laikipia County where girl school dropout is particularly high. The research examined the barriers girls face in continuing their education. The five villages in the IL Ngwesi Ranch with the highest schoolgirl dropout rates were chosen for the study. Focus groups were composed of 3-7 girls at the primary and secondary school level who were identified by teachers as being open to discussing barriers to female education in the community. Girls that had dropped out of school, both those who had returned to school and those who hadn't, were subsequently individually interviewed with a translator present. Findings indicated that lack of school fees, cultural practices including female genital mutilation and early marriage, and transactional sex that lead to unintended pregnancies were the main driving factors contributing to schoolgirl drop out in this region. Additionally, the villages that were predominately composed of the Maasai ethnic group had the highest rates of dropout. This formative research ultimately led to the establishment of a 501(c)(3) nonprofit organization, SAWA Kenya, that empowers at-risk girls living in these communities.

1032

ONE-YEAR IMPLEMENTATION OUTCOMES OF ICD-10 BASED ELECTRONIC CLINIC REPORT AT SHALOM FAMILY MEDICAL CENTER IN SANTIAGO TEXACUANGOS, EL SALVADOR

Zaira Alvarado[1], Etienne Jaime Hinojosa[2], Andrea Lopez[1], Teri Benner[3], Harold R. Garner[4], Cameron Sumpter[4], H. Dean Sutphin[2]

[1]*Shalom Family Medical Center, Santiago Texacuangos, El Salvador,* [2]*The Edward Via College of Osteopathic Medicine, Blacksburg, VA, United States,* [3]*Harvesting in Spanish, Santiago Texacuangos, El Salvador,* [4]*The Edward Via College of Osteopathic Medicine, Spartanburg, SC, United States*

A versatile low-cost user-friendly Electronic Clinical Reporting (ECR) system; using universal ICD-10 codes has potential to significantly improve global health and provide comparative data to regional PAHO and WHO. Handwritten records, poor handwriting, and lack of records in developing country low-resourced health care clinics are obstacles to accurate, efficient clinical reports and management. A US medical school Via College of Osteopathic Medicine (VCOM) developed Electronic Clinic Report (ECR) and implemented a 12-month field test (March 2019-March 2020) at the non-profit Shalom Family Medical Center in El Salvador. This North-South collaboration assessed site utilization and transferability to other developing countries utilizing implementation strategies of project manager facilitation, team development, video conferencing, physician training, consultation, and panel of experts to support report and

related product development. Data included treatment by 5 physicians of 11,491patients of which 29% were male and 71% female, in age groups of <1 (16%), 1-5 (10%), 6-18 (16%), 19- 25 (15%), 26 -35 (13%), 36- 50 (14%) > 50 (17%). Top five ICD–10 categories were Respiratory System (17%), Genitourinary system (8.5%), Endocrine and Metabolic disorders (7.4%), Circulatory System (5.9%), Parasitic diseases (4.6%). Top five medications were acetaminophen (12%), loratadine (5.2%), ibuprofen (4.6%), amoxicillin (3.7%) and rehydration formulations (3.8%). User feedback and continuous improvement facilitated enhancements in versatility; preparation for seasonal diseases; records management; efficient, accurate required monthly clinic reports (Ministry of Health, medical school and key stakeholders); program planning and development; epidemiological analyses; additional applications; and transferability to other countries. Future research on deployment to other sites is recommended along with features such as, automated pharmacy and laboratory requests (or orders) and display of laboratory data to improve and unify clinical care in a useful electronic record with access in all areas at the clinic.

1033

GLOBAL SEMINAR FOR HEALTH AND ENVIRONMENT

Dean Sutphin[1], Etienne Jaime Hinojosa[1], Gabriela Estrada[2], Sandra Gomez[2], Alba Beltre[3], Zaira Alvarado[4], Dolores Muro[5], Ana Jones[6]

[1]*Via College of Osteopathic Medicine, Blacksburg, VA, United States,* [2]*UNITEC, Tegucigalpa, Honduras,* [3]*INTEC, Santo Domingo, Dominican Republic,* [4]*Via College of Osteopathic Medicine/UEES, Blacksburg, VA, United States,* [5]*Via College of Osteopathic Medicine, Spartanburg, SC, United States,* [6]*Via College of Osteopathic Medicine, Auburn, AL, United States*

Starting in 2007, "Global Health Seminar" is a voluntary venue for medical students to examine and discuss public health issues through a case study approach among an electronic consortium of medical schools including three campuses of the Via College of Osteopathic Medicine (VCOM) in the US, Universidad Evangelica de El Salvador (UEES), Instituto Tecnológico de Santo Domingo (INTEC) in Dominican Republic and Universidad Tecnologica Centroamerica (UNITEC) in Honduras. The aim is describing features of Global Seminar as a north-south, virtual technology platform for global health education and research adaptable to current events such as COVID-19. The purpose of the Seminar is to prepare students for cross-cultural public health problem solving and team development. The Seminar February - May 16, 2020 includes 102 students: VCOM at Auburn (15), Carolinas (17), Virginia (13), UNITEC (18), INTEC (21) and UEES (18). Students engage in 3-week learning cycles to address 4 real-world cases: infectious disease, genetically modified food, population, and water; each written with a protagonist problem statement. VCOM centralized electronic platform provides cases, references, videos, learning guides, related material. Internet chat and campus resource experts assist students develop case solutions the first two weeks. On the third week, spokespersons at each campus in turn present their consensus solution in 7 minutes followed by open discussion, consensus building, and summary during a 90-minute interactive Zoom video conference. Afterwards, student independently write a case analysis of cultural, social, economic, political, environmental and medical aspects. Cross campus research such as cultural competency development is an elective option. Adaptation to COVID-19 campus closures included all zoom meetings and the addition of a "Weekly COVID-19 Pandemic Journal Log" that could become retrospective research. In conclusion, Global Seminar is an effective virtual medical education across cultural using real-world case studies, adaptable to COVID 19 conditions, and available for adaptation to other medical schools.

1034

UNDERSTANDING RESEARCH VULNERABILITIES AND RESEARCHER OBLIGATIONS ALONG THE THAI-MYANMAR BORDER

Napat Khirikoekkong[1], Suphak Nosten[1], Supa-at Asarath[2], Rose McGready[1], Francois Nosten[1], Jennifer Roest[3], Michael Parker[3], Maureen Kelley[3], **Phaik Yeong Cheah**[2]

[1]*Shoklo Malaria Research Unit, Mae Sot, Thailand,* [2]*Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand,* [3]*Ethox Centre, University of Oxford, Oxford, United Kingdom*

Research ethics guidelines set a high bar for conducting research with populations traditionally deemed vulnerable e.g. pregnant women. This results in their exclusion from important research. Our study aims to understand these vulnerabilities and identify ways of mitigating vulnerability in and through research. We conducted qualitative research around two clinical studies involving pregnant women living along the Thai-Myanmar border. Between December 2017 and January 2019 we conducted 30 semi-structured interviews and 9 focus group discussions with (1) research participants and their families (2) researchers and frontline health workers and (3) key informants. We conducted a background review of available sociodemographic and health data to characterize background vulnerabilities. We identified that participants and clinic patients experience background vulnerabilities related to: poverty, job insecurity, lack of access to healthcare, limited formal education and literacy, and uncertain legal status. We found that research has the potential to create new or exacerbate existing vulnerabilities, but also has the potential to engage participants' agency around health and to deliver important benefits. To attend research or clinic visits, participants may lose their daily wages and face travel-related risks. Participants also struggle with understanding complex study information. However, although they lead challenging lives, women have a support network that includes family, community leaders and non-governmental organisations. Research participation is often seen as a way to access healthcare. Many also demonstrate agency and resourcefulness in navigating challenges in their lives. Our findings suggest that research could exacerbate existing vulnerabilities, but also offers important benefits, including healthcare access.

1035

DEVELOPING INTERNATIONAL PARTNERSHIPS FOR MPH GLOBAL HEALTH TRAINING

Peter A. Zimmerman, Andrew Morris, **Daniel J. Tisch**

Case Western Reserve University, Cleveland, OH, United States

Case Western Reserve University (CWRU) Master of Public Health (MPH) Program has a program-wide Global Health emphasis. Global Health opportunities in all five concentrations and 11 dual degrees foster a rich diversity of student skills, perspectives, and professional impact. The training program also maintains a historical global health framework for shared training resources across 5 Schools and 9 departments, further expanding our international training expertise and partnerships. This CWRU Framework for Global Health provides the administrative and training environment to identify and develop integrated curricula, workshops, a certificate program, and mentored field experiences. These incorporate MPH students, faculty, and MPH-dual degree programs and partnerships. The MPH program recently completed a 3-year self-study and strategic planning that incorporated surveys and feedback from students, faculty, internal partners, external partners, and external reviewers as part of our re-accreditation process with Council on Education for Public Health (CEPH). Our Global Health competencies were linked with curricula, training experiences, outcomes, and partnerships. This presentation highlights our approach to establishing and supporting international training partnerships that meet the needs of our students, partners, and communities. Barriers to international training will be highlighted including program structure, mentoring capacity, language, cost, time, safety, and

travel restrictions, such as those experienced currently due to COVID-19. Lessons learned from our global health training partnership experiences will be presented.

1036

IDENTIFYING STAKEHOLDER ENGAGEMENT NEEDS FOR DEVELOPMENT, REGULATION, AND TESTING OF GENE DRIVE MOSQUITOES

Kanya C. Long, Cynthia Triplett, Omar S. Akbari, Cinnamon S. Bloss

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

Mosquitoes whose genomes have been edited to promote the rapid spread of a desired genetic trait through a population—gene drive mosquitoes—offer the potential to reduce transmission of globally important arthropod-borne viruses. However, development, regulation, and field testing of such mosquitoes will require substantial input from stakeholders. To define needs for stakeholder engagement, we interviewed scientists, policy contributors, and vector control specialists working on gene drive or other novel mosquito control methods. Interviews were semi-structured and conducted via Zoom teleconferencing. All interviews were recorded, and transcripts were analyzed using QDA Miner. Participants enrolled in the study to date (13) include representatives from academic, industry, government, and multilateral organizations. Actionable information identified as needed by study participants across professional categories included stakeholder guidance on establishing local partnerships, identifying expectations and interests early in product development, determining community-perceived risks, and increasing opportunities for cross-project collaboration. Defining engagement needs will be essential to determining possibilities for trials of gene drive mosquitoes in field settings.

1037

MARKET CHARACTERISTICS AND RISK PERCEPTION IN CAMEROON BUSHMEAT MARKETS

Karen Saylors[1], Moctar M. Mouiche[2], Ashley Lucas[1], David J. McIver[3], Annie Matsida[4], Catherine Clary[5], Victorine T. Maptue[6], Jason D. Euren[5], Matthew LeBretton[2], Ublad Tamoufe[6]

[1]*Labyrinth Global Health, Saint Petersburg, FL, United States,* [2]*Mosaic, Yaoundé, Cameroon,* [3]*Metabiota Inc., Nanaimo, BC, Canada,* [4]*Ministère de la Recherche Scientifique et de l'Innovation, Yaoundé, Cameroon,* [5]*Metabiota Inc., San Francisco, CA, United States,* [6]*Metabiota Inc., Yaoundé, Cameroon*

Behavioral practices are a key factor facilitating zoonotic disease transmission, especially in individuals who have frequent contact with wild animals, yet behaviors and practices of those who work in high-risk animal-human interfaces, such as wild animal 'bushmeat' markets in the Congo Basin are not well documented. The Congo Basin, where hunting, butchering, and consumption, of wild animal meat is frequent, represents a hotspot for disease emergence. The region has witnessed a plethora of spillover events, many of which have been traced back to close human-animal contact with bats and non-human primates. Over the last ten years, we conducted wildlife surveillance, human behavioral research, and human and animal biological sampling to identify and characterize factors associated with zoonotic disease emergence and transmission. Research was conducted through the USAID Emerging Pandemic Threats program between 2010 and 2019 including formative, qualitative studies of bushmeat markets and concurrent sampling of animals and humans, with study sites selected to maximize proximity to bushmeat markets. Sites included two hospital sites where we conducted syndromic surveillance of sick individuals with acute febrile illness, community sites where we enrolled actors of the animal value chain and bushmeat markets, where we enrolled bushmeat vendors, butchers, market managers, cleaners, and shoppers. Mixed methods research was undertaken at these sites and included investigation of bushmeat market dynamics through observational research, focus group discussions, quantitative questionnaires, and ethnographic interviews. Participants were asked about their perception of risk of zoonotic disease transmission and specific activities related to bushmeat trade, local market conditions, and regulations driving the trade of bushmeat in Cameroon. Risks associated with blood contact and infection were not well understood by most market actors. As bushmeat markets are an important disease interface, as seen with CoVID19, risk mitigation measures in markets and bushmeat alternative strategies are discussed.

1038

PRECISION MAPPING REVEALS VARIATION IN THE EPIDEMIOLOGICAL TRANSITION WITHIN MIDDLE-INCOME COUNTRIES

Nathaniel Henry

Institute for Health Metrics and Evaluation, Seattle, WA, United States

As a tool for understanding population-level transition from regimes of high childhood mortality and primarily infectious causes of death towards non-communicable causes of death, the epidemiological transition remains a valuable framework for practitioners of public health. However, the degree to which this process varies within individual countries remains incompletely understood. We investigate variation in the epidemiological transition at the district level within six middle-income countries, using spatially resolved death registration data to develop a composite metric based on geostatistical models of both child mortality and causes of death across age groups. Based on this metric, we find that progress towards regimes of low child mortality and infectious disease mortality varies substantially within countries, revealing local inequities and population-level patterns obscured by national analyses.

1039

DISTRICT HOSPITAL SYMPOSIUM: THE SOUTH AFRICAN PERSPECTIVE

Hans Jurgen Hendriks

Walter Sisulu University, Mquanduli, South Africa

The District Health System (DHS) has been adopted in SA to provide comprehensive primary healthcare. In this, district hospitals play an important role. In SA the management of PHC services were historically placed either under municipalities, the Department of Health (DoH) or non-government organisations. Over the last 15 years all PHC services have been consolidating under DoH, with management of PHC services falling to district management teams. The problem is that within the DHS, the management of PHC services is separated from the management of DHs, ironically to "protect" PHC services from the hospicentric functioning of DHs and tendency of managers and doctors to protect the hospital services at the cost of supporting PHC. Unfortunately, this only resulted in undermining the effective provision of PHC services in the DHS by creating two separate silos of management within the DHS, leading to poor integration of services, duplication of resources and personnel, and loss of general effectiveness. DHs would be the ideal placement for PHC teams and should be from where training, outreach and support, management and supervision for feeder clinics are planned and coordinated. Historical inequalities between regions are perpetuated by the infrastructure-inequality trap where regions with better absorptive capacity receiving more funding, creating more opportunities to spend the funding, worsening the inequalities. SA is ranked by the Word Bank as an "upper middle-income country", with the largest economy in Africa and spending 8.8% of its GDP on the annual healthcare budget. The population of 57 million is served by a private healthcare system serving 16% of the population with 50% of the annual healthcare budget, and a public health care system serving the remaining 84% with 45% of the annual health care budget. The SA constitution recognises the right to basic healthcare, making public healthcare free at all PHC centers as well as for all children < 6 years of age and pregnant mothers. Where other middle-income countries have made some inroads towards achieving the health related MDGs, South Africa is struggling.

1040

NEW TECHNOLOGIES AND GLOBAL STANDARDS FOR IMPROVED HEALTH OUTCOMES

Tori Ghine[1], Kaitlyn Roche[1], Nuran Idris[2], Claude Bahati[3]

[1]*GHSC-PSM, Arlington, VA, United States,* [2]*GS1 Global Office, Nairobi, Kenya,* [3]*GHSC-PSM, Yaoundé, Cameroon*

The World Health Organization estimates that one in 10 medical products in low- and middle-income countries (LMICs) is substandard or falsified [1], with 19.6 percent of reported cases representing malaria drugs [2]. Recognizing that successful health programs require reliable supplies of quality commodities, the U.S. President's Malaria Initiative's (PMI) 2015-2020 strategy includes a directive to intensify efforts to remove substandard and counterfeit drugs from the market. Tools such as GS1-compliant barcodes and the GS1 Global Data Synchronisation Network™ (GDSN®) enable a data network that is traceable across countries from manufacturer to patient level. Health workers can use GDSN data for increased treatment accountability, to verify product legitimacy, safely administer to patients, reduce medication errors, and better analyze treatments as related to patient outcomes. In parallel, cloud-based transport and temperature management and the internet of things (IoT) are using low-cost, smart technology to protect medication conditions along the supply chain and extend the reach of high-quality patient care. In 2017 at the request of PMI, the Global Health Supply Chain Program-Procurement and Supply Management (GHSC-PSM) project began introducing GS1 standards to their supply chains. By introducing these tools to health care programs, countries are benefiting from more transparent and reliable commodity supply chains, improving treatment insight for clinicians, and better serving patients. This poster will focus on the implications of developing these technologies, increasing local access to them, and the challenges and responsibilities the development community and health systems will face as they are introduced. [1] https://www.who.int/news-room/fact-sheets/detail/substandard-and-falsified-medical-products [2] https://www.who.int/medicines/regulation/ssffc/publications/GSMSreport_EN.pdf?ua=1

1041

DESIGN, DEVELOPMENT AND EVALUATION OF THE MOSQUITO REPELLENT ACTIVITY OF *AZADIRACHTA INDICA* OIL BASED SOLID LIPID NANOPARTICLES AGAINST *AEDES AEGYPTI*

Chinekwu Sherridan Nwagwu, John D. Ogbonna, Lotanna G. Nwobi, Ezinwanne N. Ezeibe, Chinenye N. Ugwu, Mumuni A. Momoh, Anthony A. Attama

University of Nigeria, Nsukka, Enugu State, Nigeria

The *Aedes aegypti* mosquito is one of the deadliest species of mosquitoes that have been known to transmit certain deadly diseases such as dengue fever, yellow fever, chikungunya amongst others. The use of repellents has shown to be a practical and economical way of preventing the diseases transmitted by mosquitoes. However, the issue of toxicity associated with the use of synthetic repellents has sparked off the need for developing safer as well as effective natural repellents. The study aimed to formulate and characterize solid lipid nanoparticulate (SLN) mosquito repellent creams using *Azadirachta indica* oil and evaluate its repellent activity against the *Aedes aegypti* mosquito. The oil used for the study was extracted from *Azadirachta indica* leaves using a soxhlet apparatus and the solid lipid nanoparticle (SLN) batches prepared using hot homogenization method. The stability of the formulated SLN creams was evaluated by measuring the pH, viscosity and particle size at time intervals. Skin irritation test, rheological studies, encapsulation efficiency, loading capacity and percentage repellence for the SLN batches were also determined. The study showed a slight decrease in the pH values and viscosity for the various formulation and an increase in particle size over time. The formulations showed no sign of skin irritation with the encapsulation efficiency values between 52 % and 71 % while loading capacity values were between 2.6 % and 10.3 %.The rheological characterization showed that the batches exhibit dominant solid-like behavior between 4°C and 40°C and that above 40°C there was an increase in the liquid-like behavior of the systems. The control (12% DEET) produced significantly higher percentage mosquito repellence compared to the SLN creams. However, the SLN formulations produced higher and more prolonged repellence than the unformulated oil extract. This study showed the performance of the SLN formulations as a possible candidate for the delivery of the *Azadirachta indica oil* for mosquito repellent application.

1042

INSECTICIDE SUSCEPTIBILITY PROFILE OF *ANOPHELES ARABIENSIS* FROM 2017-2019 IN ETHIOPIA

Meshesha Balkew Managido[1], Peter Mumba[1], Dereje Dengela[2], Gedeon Yohannes[1], Delenasaw Yewhalaw[3], Sheleme Chibsa[4], Matthew Murphy[4], Gunawardena Dissanayake[4], Jenny Carlson[5], Kristen George[5], Allison Belemvire[5], Cecilia Flatley[2], Seth Irish[6]

[1]*Abt Associates, PMI VectorLink Ethiopia Project, Addis Ababa, Ethiopia,* [2]*Abt Associates, PMI VectorLink Project, Rockville, MD, United States,* [3]*Tropical and Infectious Diseases Research Center (TIDRC), Jimma University, Jimma Town, Ethiopia,* [4]*U.S Preseident's Malaria Initiative (PMI), Addis Ababa, Ethiopia,* [5]*US President's Malaria Initiative and USAID Bureau for Global Health, Office of Infectious Disease, Malaria Division, Arlington, VA, United States,* [6]*US President's Malaria Initiative and Entomology Branch, Center for Disease Control and Prevention, Atlanta, GA, United States*

As part of entomological monitoring activities, the President's Malaria Initiative VectorLink Ethiopia project conducted insecticide susceptibility tests from 2017 to 2019 in selected sentinel sites to generate data to inform vector control decisions. Populations of *Anopheles arabiensis* were investigated for their susceptibility to pirimiphos-methyl, bendiocarb, propoxur, deltamethrin, permethrin, alpha-cypermethrin and clothianidin using WHO tube tests, and chlorfenapyr using CDC bottle bioassays. Resistance intensity was evaluated where resistance was observed. Piperonyl butoxide (PBO) and molecular assays were conducted to assess mechanisms of resistance. Tests were conducted on wild mosquitoes raised from larvae collected from 12 sites in 2017, 13 in 2018, and 21 in 2019. Susceptibility to pirimiphos-methyl was observed in all 21 sites; susceptibility to bendiocarb and propoxur was recorded in 20/21 and 19/21 sites, respectively. *Anopheles arabiensis* was susceptible to clothianidin and chlorfenapyr in 8/8 sites tested. *Anopheles arabiensis* was resistant to all three pyrethroids tested in 21/21 sites. The results of phenotypic resistance tests indicated that resistance intensity to the three pyrethroids was not constant between sites or within individual sites over time. Pre-exposure to PBO restored susceptibility to deltamethrin and alphacypermethrin in 13/13 sites and to permethrin in 11/13 sites, indicating the presence of metabolic resistance mechanisms. In 2018, the frequencies of knockdown resistance (*kdr-west*) genotypes were 54.3% and 34% homozygous and heterozygous resistant genotypes, respectively, indicating likely involvement of target site resistance. In conclusion, the populations of *An. arabiensis* are susceptible to insecticides commonly used for IRS, but resistant to pyrethroids, possibly due to metabolic and target site resistance mechanisms. Robust insecticide resistance management strategies should be implemented while the vector remains susceptible to third generation insecticides to mitigate any further development of insecticide resistance.

1043

ALLELIC FREQUENCIES OF KDR AND ACE-1 MUTATIONS AMONG WILD *ANOPHELES ARABIENSIS* AND *AN. MELAS* POPULATIONS IN THE COASTAL ZONE OF LOW MALARIA TRANSMISSION IN SENEGAL

Ousmane Sy

UCAD, Dakar, Senegal

In central western Senegal, malaria transmission has become weak due to several effective interventions. The residual transmission that takes

place in the area is mainly ensured by *An. arabiensis* and *An. melas*. The interruption of certain control measures could lead to a resurgence of the disease and spread of insecticide resistance mutation such as Kdr and Ace-1R, in the main malaria vectors. Therefore it becomes urgent to assess the levels and mechanisms of insecticides resistance in vectors involved in residual transmission. The malaria vector populations was collected in each of the three selected villages using overnight human landing catches (HLC) and pyrethrum spray catches (PSC). The mosquitoes collected were identified to species level and molecular forms and then genotyped for the presence of L1014F-kdr, L1014S-kdr and ace-1R mutations using qPCR. *An. arabiensis* and *An. melas* were the most representative species of the Gambiae complex in the study area with 69.4% and 28% respectively. The allelic frequency of kdr-east was relatively higher than kdr-west mutation in *An. arabiensis* population with respectively 22.66% and 9.96%. Overall, the frequencies of both kdr mutations were very low in *An. melas* population with 1,12% for kdr east and et 0,4% for kdr west. The G119S mutation had been found as heterozygote form only in *An. arabiensis* population in all investigated sites with global frequency of 2% and was completely absent in *An. melas* population. The presence of the kdr mutation in *An. arabiensis* and *An. melas* populations the main vectors ensuring the residual transmission of malaria in the hotspots in central-western Senegal constitutes a potential risk which can compromise the effectiveness of vector control measures. The monitoring of this resistance is becoming a priority to achieve the goal of elimination in this part of Senegal. The detection of *ace-1* mutation in *An. arabiensis* population for the first time in Senegal should also be confirmed and monitored.

1044

HOUSEHOLD ACCEPTANCE OF YEAST INTERFERING RNA BAITED OVITRAPS IN TRINIDAD

Akilah Stewart[1], Nikhella Winter[1], Jessica Igiede[2], Rachel M. Wiltshire[3], Limb K. Hapairai[3], Azad Mohammed[1], David W. Severson[2], Molly Duman-Scheel[3]

[1]*The University of the West Indies at St. Augustine, Trinidad and Tobago, St. Augustine, Trinidad and Tobago,* [2]*The University of Notre Dame, Notre Dame, IN, United States,* [3]*Indiana University School of Medicine, South Bend, IN, United States*

Diseases resulting from Zika, dengue, chikungunya and yellow fever arboviral infections remain a major public health burden in Trinidad. *Aedes* mosquitoes, the primary vectors for transmission of these viral pathogens, continue to flourish due to local challenges in vector control. These mosquitoes lay eggs in water-filled containers located in close proximity to humans. This preference for container breeding sites provides the opportunity to both monitor and control these mosquitoes through the use of larval lethal ovitraps, dark colored containers filled with water and attractants to lure gravid female mosquitoes and kill their offspring. We recently designed a yeast-based interfering RNA baited ovitrap, a novel approach that circumvents general issues relating to insecticide resistance and which poses no threat to non-target organisms. Following a general evaluation of local participants' knowledge of mosquitoes, mosquito control practices, and feelings about the new technology, which were assessed using paper surveys and community engagement forums, a small-scale field trial using baited-ovitraps was undertaken. The trial was performed in Curepe, a community located in north Trinidad, where ovitraps were placed throughout nine discrete blocks in the yards of participants. During the course of the trials, formal interviews were conducted with homeowners to assess their acceptance of the intervention. These interviews revealed that the participants had a general understanding of mosquito-borne illnesses and engaged in some form of mosquito control. Having perceived a general reduction in mosquitoes on their properties during the trials, most participants indicated they were willing to purchase the new ovitraps if they were shown to be safe and affordable. Others offered useful advice concerning product design, distribution, application, and the need for further mosquito educational campaigns. These results, in combination with paper survey and community engagement forum data, suggested that local stakeholders were willing to augment existing control strategies through the addition of RNAi-based yeast ovitraps.

1045

INSECTICIDE RESISTANCE STATUS AND MECHANISMS IN *AEDES* MOSQUITOES IN GHANA

Anisa Abdulai, Simon Kweku Attah, Akua Obeng Forson, Christopher Mfum Asenso, Yaw Asare Afrane

University of Ghana, Accra, Ghana

Aedes mosquitoes are vectors of dengue and yellow fever in Ghana which are major public health concerns. Widespread insecticide resistance in the vectors further complicates control. Thus, prior knowledge about the mechanisms of resistance in *Aedes* populations in Ghana will be useful in improving pre-existing arboviral vector control measures in order to control and prevent any future outbreaks. This study was carried out in the urban sites (Accra and Tema) and suburban sites (Navrongo and Ada). *Aedes* larvae were collected from study sites and raised to adults in the insectary. Phenotypic resistance was determined using WHO susceptibility tests and resistant genes were detected using allele-specific PCR. A synergist assay was performed with piperonyl butoxide (PBO) to detect the involvement of oxidase enzymes in resistance. The results showed high phenotypic resistance to DDT (11.3% to 75.8%) and pyrethroids (62.5% to 88.8%) in all sites. *Aedes* mosquitoes in Tema were resistant to all classes of insecticides tested. Suspected resistance to carbamate and organophosphates was detected in some sites. High frequency of point mutation at the voltage-gated sodium channel (F1534C) was detected in resistant Aedes mosquitoes from all sites. Pre-exposure to PBO significantly enhanced the susceptibility of Aedes to some of the insecticides tested. This may be an indicator that metabolic enzymes (oxidases) may be involved in the development of resistance in some *Aedes* populations. These finding suggests that resistance profiles in Aedes mosquitoes vary across Ghana and are mediated by different mechanisms. Thus, there is a need for regular monitoring of resistance for the control of arboviral diseases in Ghana.

1046

A COMPARATIVE ANALYSIS OF THE SPATIAL DISTRIBUTION OF INSECTICIDE RESISTANCE IN *AEDES AEGYPTI* AND *AE. ALBOPICTUS* FOR INFORMED CONTROL OF ASSOCIATED NEGLECTED TROPICAL DISEASES

Duncan K. Athinya[1], Melinda P. Hadi[2], Seline A. Omondi[3], Eric O. Ochomo[3]

[1]*Vestergaard, Nairobi, Kenya,* [2]*Vestergaard, Lausanne, Switzerland,* [3]*Kenya Medical Research Institute, Kisumu, Kenya*

Forecasted impacts of climate change indicate expansion of the distribution of *Aedes aegypti* and *Ae. albopictus,* and associated neglected topical diseases such as dengue fever, chikungunya, Zika, and yellow fever. Control of these diseases in epidemics relies on insecticidal vector control. Knowledge of the spatial distribution of insecticide susceptibility and resistance in *Ae. aegypti* and *Ae. albopictus* is essential in informing the deployment of insecticidal tools. Data for this analysis was from IR Mapper, a free online tool that maps insecticide resistance in *Ae. aegypti* and *Ae. albopictus,* in addition to *Anopheles* species. We compared insecticide resistance trends in *Ae. aegypti* and *Ae. albopictus*. Data collected between 2010 and 2019 was spatially aggregated by continent, country and first administrative boundaries and compared for the two species. There were 4774 tests of both phenotypic and genotypic insecticide resistance in both adult and larval stage *Ae. aegypti* and *Ae. albopictus*. The tests were from 791 localities in 229 first administrative boundary areas in 49 countries within 6 continents. *Ae. aegypti* (63.5%) was more frequently investigated than *Ae. albopictus* (36.5%). Data where both species were investigated was available from 32 first administrative level boundary areas in 13 countries. Confirmed resistance to pyrethroids was more frequently reported in *Ae. aegypti* (73%) than in *Ae. albopictus*

(61.5%) while confirmed resistance to organophosphates was more frequently reported in *Ae. albopictus* (42.9%) than in *Ae. aegypti* (23.8%). Over expression of esterases, oxidases and GSTs were involved in observed resistance for both species. There were some specificity in knockdown resistance mutations involved in resistance between the species; however, this could be due to testing bias. IR Mapper is a useful tool for identifying resistance data gaps especially in areas where epidemics are most likely to occur and informing deployment of insecticidal vector control tools.

1047

INFLUENCE OF AGRICULTURAL PRACTICES ON THE PATHWAYS OF METABOLIC RESISTANCE IN MALARIA VECTORS

France Paraudie Affoue Kouadio[1], Nadja Christina Wipf[2], Angele Sika Nygble[3], Behi Fodjo Kouadio[1], Christabelle Gba Sadia[1], Konstantinos Mavridis[4], John Vontas[5], Pie Müller[2], Chouaïbou Seïdou Mouhamadou[6]

[1]*Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, Université Nangui Abrogoua, Abidjan, Côte D'Ivoire,* [2]*Swiss Tropical and Public Health Institute and University of Basel, Basel, Switzerland,* [3]*Université Nangui Abrogoua, Abidjan, Côte D'Ivoire,* [4]*5Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion, Greece,* [5]*Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion and Agricultural University of Athens, Athens, Greece,* [6]*Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC, United States*

Insecticide-based vector control is facing an emerging resistance of malaria vectors to the major public health insecticides. Recent studies suggest that pesticide use in agriculture is a selection pressure for resistance in mosquitoes. Our study focuses on monitoring insecticide resistance in malaria vectors in relation to agricultural practices and investigates the resistance mechanisms involved. Mosquito larvae (*Anopheles gambiae s.l.*) have been collected in southern Côte d'Ivoire at three field sites with high agricultural pesticide use including two rice-growing areas (Agboville, Tiassale) and a vegetable growing area (Dabou). Insecticide susceptibility of adult mosquitoes was assessed using standardized WHO tube tests. High phenotypic resistance to all classes of insecticides tested (<25% mortality) was observed at all sites; however, 100% mortality was observed with malathion in rice growing areas. We used multiplex TaqMan RT-qPCR to characterize the underlying molecular resistance mechanisms in 50 individual mosquitoes per site. The L1014F target-site mutation was found in all sites with an allelic frequency of 67% in Tiassalé, 64% in both Agboville and Dabou. We also measured the gene expression of eight targets implicated in metabolic insecticide resistance relative to the house-keeping gene RPS7. CYP4G16 gene expression was greater in Dabou (vegetable growing area) with an average relative quantification unit (RQ) three times (75.05%) higher than in Tiassalé (rice growing area; 24.27%) compared to 12.38% for the susceptible strain Kisumu (P<0.001). As for the CYP6M2 gene, its RQ was two times higher in the rice-growing areas Tiassalé (13.9%) and Agboville (13.88%) than in the vegetable growing area Dabou (7.41%) versus 0.33% for Kisumu (P<0.001). Finally, CYP6Z1 was overexpressed in both growing areas, while the RQ was highest in Agboville (56.75% versus 4.55% for Kisumu, P<0.001). The current study shows a fairly similar trend in target site mutations and variation in the metabolic pathways involved in this resistance at different sites. These results should be used to support the vector control program.

1048

SOME SYSTEMIC VETERINARY DRUGS USED IN LIVESTOCK AND DOGS TO CONTROL TICKS CAN ALSO BE USED AGAINST *ANOPHELES* MALARIA VECTORS

Jefferson A. Vaughan[1], Todd Molden[2], Marc Bauer[2], Staci M. Dreyer[1]

[1]*University of North Dakota, Grand Forks, ND, United States,* [2]*North Dakota State University, Fargo, ND, United States*

Many malaria vector control programs rely on indoor residual spraying and insecticide-treated bed nets. This is effective against vector species that feed indoors at night and rest inside the house afterwards. However, many malaria vectors in Asia and the Americas exhibit different behaviors – i.e., they are exophagic (*i.e.,* bite outdoors), exophilic (*i.e.,* remain outdoors after feeding), and zoophagic (*i.e.,* as likely to feed on non-humans as humans). Malaria elimination in these regions may require other tactics. This study investigated whether various veterinary drugs used to treat livestock and dogs for ticks were also effective against zoophagic malaria vectors. Using membrane feeding techniques, comparative toxicities (expressed as oral LC-50s) and effects on mosquito fecundity were determined for various compounds after they were fed to *Anopheles albimanus* and *An. stephens*i mosquitoes. Four compounds – ivermectin, fipronil, fluralaner, afoxolaner – exhibited acceptable toxicity profiles against the target mosquito species (i.e., the oral LC-50 values were lower than peak plasma concentrations reported for treated host animals) to warrant further investigation as candidates for use in the field. In addition, two avermectin compounds (*i.e.*, ivermectin and abamectin) and the phenypyrazole compound, fipronil, inhibited ovarian development and significantly reduced fecundity of surviving mosquitoes. The isoxazoline compounds did not alter ovarian development or affect fecundity.
Using dairy calves as experimental animals, two commercially available formulations of ivermectin – injectable and topical "pour-on" – were used to compare the duration of mosquitocidal activities when applied at the recommended dose. The injectable formulation killed *An. stephensi* for up to 14 days after treatment. The topical formulation killed *An. stephens*i up to 23 days after treatment. These results suggest that judicious treatment of livestock with commercially available systemic acaricides, such as fipronil or ivermectin, could be a useful tool in the elimination of residual malaria transmission by zoophagic vectors.

1049

COMMUNITY BASED APPROACH TO CONDUCT WIDESPREAD MOSQUITO SURVEILLANCE USING BIOGENTS GRAVID *AEDES* TRAPS IN PHNOM PENH, CAMBODIA

Jodi M. Fiorenzano[1], Didot Prasetyo[2], Rithea Leang[3]

[1]*US Navy, Sembawang, Singapore,* [2]*AC Investment Company, Phnom Penh, Cambodia,* [3]*National Center for Parasitology, Entomology, and Malaria Control, Phnom Penh, Cambodia*

Dengue disease is endemic throughout the tropical and sub-tropical world, is responsible for over 100 million cases annually, and is transmitted mostly via infected *Aedes* mosquitoes. The dengue burden in Southeast Asia has been well established for over 75 years, including in Cambodia where dengue outbreaks occur frequently. Historically, all four dengue serotypes co-circulate in Cambodia, increasing the risk of serve dengue infection. The National Center for Parasitology, Entomology and Malaria Control (CNM) is lead for mosquito control and surveillance in Cambodia. CNM's integrated vector management practices conduct small-scale mosquito surveillance in response to dengue cases. Currently, CNM lacks the equipment and ability to conduct wider scale *Aedes* mosquito surveillance. In order to enhance adult mosquito surveillance, and mitigate dengue spread, NAMRU-2 and CNM conducted a pilot in Phnom Penh, whereby 12 volunteers were recruited in the vicinity of Phnom Penh to place passive adult mosquito surveillance devices: BioGents *Aedes* Gravid traps (GATs) in and around their houses, and to collect the sticky card insert weekly. During the 14-week study, 2,018 adult mosquitoes were collected; 61.8% *Aedes*, 37.4% *Culex*, <0.1% Anopheles, and 0.5% others. Through the

duration of this pilot study, the utility of the GAT and the ability of the volunteers to aid CNM in mosquito surveillance paved the way for CNM and NAMRU-2 to expand the mosquito surveillance to ten additional health centers. The ability to document where the mosquitoes are through simple means may lead to more proactive verse reactive mosquito control. Additional surveillance must be conducted over a longer period of time and at more sites to be able to determine sustainability of this surveillance in Cambodia. Future research can establish if, this adult *Aedes* mosquito surveillance technique could be applicable to other low-income countries in Southeast Asia were there is little to no access to battery operated traps or costly mosquito surveillance baits.

1050

MULTIPLE INSECTICIDES RESISTANCE AND FIRST DETECTION OF *ACE*-1^R MUTATION IN *CULEX QUINQUEFASCIATUS* FROM LAGOS, NIGERIA

Ifeoluwa Kayode Fagbohun[1], Emmanuel Taiwo Idowu[1], Tolulope Amos Oyeniyi[2], Adedapo Adeogun[2], Taiwo Sam Awolola[2], Olubunmi Adetoro Otubanjo[1]

[1]*University of Lagos, Lagos, Nigeria,* [2]*Nigerian Institute of Medical Research, Lagos, Nigeria*

The control and management of *Culex quinquefasciatus* borne diseases heavily rely on the use of insecticides based measures but the emergence and spread of insecticides resistance had been the cause of challenge and led to major setback in recent gains against the control of mosquito borne diseases. Here we assessed the susceptibility status of *Cx. quinquefasciatus* to three different classes of neurotoxic insecticides and also determined the possible resistance mechanisms. Culex were collected from Alimosho, Kosofe, Badagry and Ibeju-Lekki Local Government Areas (LGAs) of Lagos State. Adult female exposed to DDT (4%), permethrin (0.75%), and PBO (4%) synergized assays using WHO procedures. Resistance mechanisms were assessed using molecular and biochemical techniques. Resistance to DDT and permethrin to Cx. quinquefasciatus was recorded in all the LGAs with 24hrs percentage mortality ranging from 5% to 86% Resistance to bendiocarb was also recorded in Alimosho (7%) and Kosofe (19%) LGAs and possible resistance (96%) in Ibeju-Lekki LGA. PBO synergists was able to reduce the KDT50 and KDT95 of PBO synergized bioassays were significantly reduced compared to the non-synergized bioassay. Heterozygote resistant Ace1R gene was detected in Cx. quinquefasciatus population from Kosofe and Alimosho, kdr L1014S and L1014F were not detected in this study. The activities of Cytochrome P450 monooxygenase and glutathione S-transferase detoxifying enzymes negatively correlates with 24hrs percentage mortality of Cx. quinquefasciatus. Resistance reported in Cx. quinquefasciatus to multiple classes of insecticides may result in difficulty in control of lymphatic filariasis in these areas.

1051

LARVICIDAL ACTIVITY OF SOME NIGERIAN PLANTS EXTRACTS AND THEIR PARTITIONED FRACTIONS AGAINST *CULEX QUINQUEFASCIATUS*

Francis Banjo Adewoyin, Funmilayo G. Famuyiwa, Oluyemi J. Oladiran, Oluwatosin R. Obagbemi

Obafemi Awolowo University, Ile-Ife, Nigeria

The methanol extracts of fifteen plants and their partitioned fractions were individually screened for larvicidal activity against *Culex quinquefasciatus,* the vector of lymphatic filariasis with a view to identifying the active ones. After solubilising in Tween 80, different concentration (1, 2, 3, 4, 5 mg/mL) of the fractions were tested against mosquito larvae in five (5) replicates using 25 larvae per concentration. Positive control received methanol leaf extract of *Nicotiana tabacum.* About fifty six percent (56.3 %) of the tested fractions had moderate larvicidal activity. The fruit extract of *Thevetia neriifolia* and the leaf extracts of *Calotropis procera* and *Solanum macrocarpon* were the most active. Each of the extracts had one or two highly active partitioned fractions after 48 hours but the n-hexane fractions of *S. macrocarpon* (0.78 ± 0.03 mg/mL) and *Spondias mombin* (0.81 ± 0.03 mg/mL) were the most active. The percentage mortalities were determined after 24 and 48hours of exposure and the LC_{50} and LC_{90} values were predicted using Microsoft Excel program 2010. Further purification of the highly active fractions of these extracts could lead to the isolation of potent larvicidal compounds that could be used in the control of *Cx. quinquefasciatus* mosquito.

1052

EMERGING ROLE OF N1575Y MUTATION IN PYRETHROID RESISTANT *ANOPHELES GAMBIAE* S.L. IN NIGERIA

Tolulope Amos Oyeniyi

Nigerian Institute of Medical Research, Lagos, Nigeria

In view of recent findings that codon 1575 mutation increases the L1014F mediated resistance in *Anopheles* mosquitoes, we investigated the presence of this mutation in four sites with intense pyrethroid resistance in Nigeria. Permethrin and deltamethrin susceptibility test was carried out using 2-3 days old adult *Anopheles* from larval collections. Based on the susceptibility test result, intensity tests with 5x and 10x concentrations were carried out at sites with high resistance (% mortality>t85). Resistant specimens were identified by PCR and screened for the presence of L1014F and N1575Y mutations using allele specific PCR and Taqman assay respectively. The frequency of *kdr* mutation for permethrin varied from 0.71-0.75 for 5x and 0.73-0.75 for 10x. The *kdr* frequency for deltamethrin was significantly low: 0.54-0.60 for 5x and 0.66-0.71 for 10x. Percentage increase in *kdr* frequency between 1x and 5x permethrin doses ranges from 1.4% to 11.9% in all sites except Edo and Anambra; while Lagos had 1.4% between 5x and 10x. For deltamethrin, Lagos and Niger had the same (3.8%) percentage increase in *kdr* frequency between 1x and 5x, while 5x and 10x was as high as 23.6% and 31.5% respectively. Resistant *Anopheles gambiae* at 5x and 10x concentrations of permethrin showed the presence of homozygous (Y) and heterozygous (NY) mutant allele in two sites. From this study, the mutation has no significant association with resistance intensity (χ^2=0.8, P=0.3711). There is need for frequent monitoring to forestall the effect this may have on national malaria control programs.

1053

IMPACT OF HUMAN PRACTICES ON INSECTICIDES RESISTANCE IN *ANOPHELES GAMBIAE* IN ELIBOU, SOUTHERN CÔTE D'IVOIRE

Bedjou Prisca N'DRI

Centre Suisse de Recherches en Cote d'ivoireand Swiss Tropical Public Health Institute, Abidjan, Côte D'Ivoire

Increasing insecticide resistance in anopheline mosquitoes threatens the success of insecticide-based malaria vector control relying on insecticide-treated nets and indoor residual spraying. Yet, it remains unclear what the underlying processes of selection are. A hypothesis is that insecticides other than those used for public health may drive the selection process. Following the characterisation of molecular and phenotypic insecticide resistance, we interviewed inhabitants of Elibou, a village in southern Côte d'Ivoire, about their knowledge, attitudes, practices and beliefs (KAPB) about the use of insecticides in both agriculture and public health. To determine the resistance status of the local *Anopheles* population, we collected larvae and exposed the emerged adults to diagnostic concentrations in WHO insecticide susceptibility tests, including a pyrethroid (deltamethrin), an organophosphate (malathion), a carbamate (bendiocarb) and DDT. In addition, we extracted DNA from the mosquitoes and ran diagnostic PCRs to identify loci associated with insecticide resistance and for species identification. We found that *Anopheles gambiae* s.s. and *A. coluzzii* are the predominant local malaria vectors and that both species are resistant to all four insecticides tested. In parallel, we identified the presence of L1014S and N1575Y mutations in the voltage-gated sodium channel conferring resistance to pyrethroids. Half of the interviewees mentioned the general use of agricultural insecticides, and the majority of those stated they would also use these products at

home, including pyrethroids and organophosphates. Our results suggest that insecticides with similar mode of actions are being used off label for multiple purposes, potentially driving resistance against public health insecticides

1054

EFFECT OF YEAST-BASED DELIVERY OF MICROENCAPSULATED ESSENTIAL OIL LARVICIDE ON LARVAL STAGES OF *ANOPHELES* MOSQUITOES

Brian Medernach[1], Pablo Silva[2], Yash Gupta[2], **Prakasha Kempaiah**[2], Justin Harbison[2], Stefan Kanzok[3], Ravi Durvasula[2]

[1]*Loyola University Medical Center, Chicago, IL, United States,* [2]*Loyola University Chicago Stritch school of Medicine, Maywood, IL, United States,* [3]*Loyola University Chicago, Chicago, IL, United States*

According to the WHO, insect borne illness accounts for 17% of all infectious diseases with an estimated 700,000 deaths globally. There are an estimated 230 million cases of malaria annually which account for over 400,000 mosquito borne illness related deaths/year. The control and eradication strategies against malaria include prevention, treatment, vaccine development and vector control. Vector control; specifically synthetic larvicide use has described disadvantages due to toxicity to humans, toxicity to environment and non-target species, cost and concern for the evolution of target resistance. Essential oils have known larvicidal properties and also are demonstrated to be non-toxic to humans. Prior research has demonstrated activity of yeast encapsulated orange oil (OO) larvicide activity against several larval stages of *Aedes aegypti* under laboratory conditions. Here we report on yeast encapsulated essential oil larvicide activity against the larval stages of Anopheles stephensi, owing to a significant burden of disease from *Anopheles* sp. transmitted illness and death from malaria. Encapsulated oil particles were prepared following an established protocol that utilizes food-grade orange oil (OO) and baker's yeast cells . Larvicidal testing was performed at Loyola University Chicago insectary using larvae of *A. stephensi*. Once larvae reached the desired stage, they were placed into cups containing 100 mL deionized (DI) water and varied concentrations of larvicide. Larvicide quantitative bioassays were performed using 1st (L1), 2nd (L2), 3rd (early L3 and L3) and late 3rd/early 4th (L3/L4) instar larvae. Each cup contained 25 larvae and each dose was replicated on 4 cups for a total of 100 larvae per trial. Live and dead larvae were counted after 24 h of larvicide exposure to determine mortality rate at each concentration. This test was replicated three times to determine mortality rates and lethal dose (LD) values calculated using the logit generalized linear model implemented in R. All lethal doses are presented with confidence interval at 95% CI. The study is still ongoing and results will be compiled throughout the year.

1056

GENETIC BASIS OF INDOOR AND OUTDOOR-RESTING BEHAVIOR OF MALARIA VECTORS IN NORTHERN GHANA

Majidah Hamid-Adiamoh[1], Karim Mane[2], Davis Nwakanma[2], Umberto D'Alessandro[2], Yaw Afrane[3], Alfred Amambua-Ngwa[2]

[1]*West African Centre for Cell Biology of Infectious Pathogens (WACCBIP), Accra, Ghana,* [2]*Medical Research Council Unit, The Gambia at the London School of Hygiene & Tropical Medicine, Banjul, Gambia,* [3]*Department of Medical Microbiology, College of Health Sciences, University of Ghana, Accra, Ghana*

The emergence of behavioral and genetic divergence in vector populations has been attributed to prolonged exposure to insecticidal interventions. Towards characterizing the genetic basis underlying indoor and outdoor-resting behavior of *Anopheles gambaie* sl populations in Northern Ghana, we performed amplicon sequencing of six genes; cytochrome P450, voltage sodium channel (Vgsc), gustatory receptors GPRgr13 and GPRor38 as well as olfactory receptors GPRor 69 and GPRor70, which have been previously associated with behavioral and ecological segregation in sibling members of *An. gambiae* complex. The overall genetic diversity (Heterozygosity (He and Ho) and nucleotide diversity (ϖ)) within and between mosquito populations was low across all gene loci (mean Ho = 0.1-0.4±0.01-0.1; mean He= 0.1-0.3±0.01-0.07; Mean ϖ= 0.06-0.14, P>0.05). However, we found strong evidence of differentiation (F_{ST}) in cytochrome P450 between mosquito populations despite low level of gene flow (Nm) and inbreeding (F_{IS}) (F_{ST} =0.38-0.72, P <0.001; F_{IS}= -0.06-0.49, P=0.8; Nm=0.1-0.4; Analysis of percentage variance components (AMOVA) =58%). Indeed, indoor and outdoor sub-populations significantly clustered separately on principal coordinate analysis (PCoA) of variants at this locus. Differentiation between populations and species were also evident from variation in other genes (Analysis of percentage variance components (AMOVA) =32-67%). Thus, this study detected gene flow and P450-driven genetic structure among *Anopheles gambiae* sl populations. The spread of insecticide resistance traits could present a challenge to the effectiveness of vector control measures in the study area. Additional investigations are required to determine optimal intervention tools for effective targeting of both indoor and outdoor-resting vectors in the study region.

1057

POPULATION-LEVEL DEMONSTRATION OF A CONFINABLE CRISPR/CAS9 GENE DRIVE SYSTEM IN THE YELLOW FEVER MOSQUITO *AEDES AEGYPTI*

Michelle A. Anderson[1], **Estela Gonzalez**[1], Matt Edgington[1], Joshua Ang Xin De[1], Katherine Nevard[1], Lewis Shackleford[1], Sebald Verkuijl[1], Tim Harvey-Samuel[1], Phil Leftwich[2], Luke Alphey[1]

[1]*The Pirbright Institute, Pirbright, United Kingdom,* [2]*University of East Anglia, Norwich, United Kingdom*

Prominent amongst insect vectors of human disease is the yellow fever mosquito *Aedes aegypti*. This species is considered the primary vector for a range of important arboviruses including dengue, yellow fever, chikungunya, and Zika. First proposed by Austin Burt in 2003, the 'Homing Endonuclease' gene drive has gained wide-spread attention as a means of spreading a trait of interest or a genetic load through a target pest population, with much focus placed on their potential for mosquito control. The highly targetable nature of the CRISPR/Cas9 system has been recognised as advantageous in the engineering of such gene drives as it can be easily adapted to target different genomic sequences. In particular, the ability to engineer potentially geographically confineable gene drive systems is highly desirable and may be a prerequisite for the wide-spread use of these technologies in the field. The homing process and components for building CRISPR/Cas9 gene drives have been demonstrated in a variety of dipterans including the model organism *Drosophila melanogaster* and the disease vectoring mosquitoes *Anopheles gambiae*, *Anopheles stephensi* and *Ae. aegypti.* However, whilst the spread of these systems has been trialled at the population-level in *D. melanogaster* and the *Anopheles* species such empirical demonstration is lacking in *Aedes aegypti*. Additionally, to date, no empirical population-level assessment of a confinable 'split-drive' design has been conducted in any insect. Using regulatory sequences from the *Ae. aegypti Benign gonial cell neoplasm* (*bgcn*) homologue to drive germline Cas9 expression and a target homing cassette integrated into the *Ae. aegypti Kynurenine 3-monooxygenase* (*kmo*) gene we demonstrate empirically the ability of CRISPR/Cas9 homing drives to increase in frequency within *Ae. aegypti* populations and confirm that split-drive designs conform to modeled predictions on their behaviour and spread over multiple generations.

1058

THE EVOLUTION AND EXPRESSION OF GUSTATORY RECEPTORS IN THE *ANOPHELES GAMBIAE* COMPLEX OF MALARIA VECTORS

Zachary R. Popkin-Hall, Michel A. Slotman

Texas A&M University, College Station, TX, United States

Mosquitoes primarily use chemosensation to locate vertebrate hosts and plant nectar sources. The chemosensory gene repertoire in insects contains four major gene families: the gustatory receptors (*Grs*), the olfactory

receptors (*Ors*), the ionotropic receptors (*Irs*), and the odorant binding proteins (*Obps*). Numerous studies have shown positive selection at the molecular level on chemosensory genes involved in host adaptation. In this study, we investigated the molecular evolution of *Grs* within the *Anopheles* (*An.*) *gambiae* complex. Sequences for 60 *Grs* from six of the nine complex species were extracted from data produced by the 16 *Anopheles* genomes project. These data were analyzed using the McDonald-Kreitman, Tajima's D, Fay and Wu's H, joint DH, and E tests. We are particularly interested in genes that show positive selection when comparing the anthropophilic malaria vectors *An. coluzzii* and *An. gambiae* s.s. against the zoophilic non-vector *An. quadriannulatus*. *Gr18* and *Gr50* show a significant excess of fixed replacement substitutions between one or more anthropophilic species and *An. quadriannulatus*. However, these *Grs* are expressed at low levels in chemosensory organs. *Gr48* and *Gr60*, which are expressed at higher levels in the proboscis and maxillary palps, respectively, of male *An. quadriannulatus*, also show a marginally significant excess (p=0.054 and p=0.053, respectively). Furthermore, *Gr17*, a highly expressed sugar receptor recently underwent a selective sweep in *An. coluzzii*, as did the lowly-expressed *Gr19* and *Gr41* in *An. quadriannulatus*. Finally, three *Grs* in *An. gambiae* s.s. show evidence of recovery from a selective sweep, most interestingly a CO_2 receptor, *Gr24*. We have identified four *Grs* undergoing positive selection, and six that show evidence of selective sweeps. While the biological significance of most of these genes is unclear due to their low expression in chemosensory tissues, the role of *Gr48* and *Gr60* in male *An. quadriannulatus* merit further investigation.

1059

A PHYSICAL MAP OF THE *AEDES ALBOPICTUS* GENOME

Reem A. Masri[1], Atashi Sharma[1], Dmitriy A. Kargodin[2], Jeremy Jenrette[1], Zhijian Tu[1], Mariangela Bonizzoni[3], Maria V. Sharakhova[1]

[1]*Virginia Tech, Blacksburg, VA, United States,* [2]*Federal Research Center Institute of Cytology and Genetics, Novosibirsk, Russian Federation,* [3]*University of Pavia, Pavia, Italy*

The Asian tiger mosquito, *Aedes albopictus* is an aggressive, day-time biting insect that is emerging throughout the world as a public health threat following its primary role in recent dengue (DENV) and Chikungunya (CHIKV) outbreaks. Part of its impact on human health is due to its ability to adapt to suburban and urban environments, develop egg diapause when exposed to short day length, and its opportunistic blood choice (preferring to bite humans if available). Progress in understanding the science of mosquitoes and building up the instruments to battle this species has been eased back by the absence of a great genome assembly. In this study we developed a new approach using fluorescence in situ hybridization to validate and orient fifty transcripts or gene exons from twenty largest genomic supercontigs to the mitotic chromosomes of Ae. albopictus. Physical mapping allows to check for the quality of the assembled genome by confirming or dismissing assembly predictions. Our physical map covered 57% of the genome assembly by targeting nineteen of the largest genomic scaffolds and five of the minor ones. Our approach was based on amplification of DNA probes using cDNA instead of Bacterial Artificial Chromosome (BAC) clones because utilizing BAC clones for highly repetitive genomes such as Ae. albopictus becomes extremely challenging. In addition, we mapped 18S rDNA in the region of the secondary constriction in region 1q22. The intensity of the signal significantly varied among chromosomes suggesting variations in number of ribosomal genes. We also mapped the largest bioinformatically-identified viral integration in the assembly confirming that this viral DNA is integrated to the chromosome 2q close to the telomere end. Physical mapping of polyphenol oxidase genes supported the genomic data of the presence of three clusters of these genes in chromosome 2. The availability of the improved reference genome assembly for arboviral vector Ae. albopictus will stimulate further genetic studies aimed at preventing mosquito-borne disease transmission.

1061

DISTRIBUTION AND BIONOMICS OF *AEDES AEGYPTI* IN URBAN AREAS OF SOMALI REGION, EASTERN ETHIOPIA

Solomon Yared Gebremeskel[1], Araya Gebresilassie[2], Tamar Carter[3]

[1]*Jigjiga University, Jigjiga, Ethiopia,* [2]*Addis Ababa University, Addis Ababa, Ethiopia,* [3]*Baylor University, Waco, TX, United States*

Arboviral diseases such as yellow fever, dengue, and Chikungunya are of public health importance in Ethiopia. Dengue and Chikungunya diseases have been arisen outbreak frequently in Somali Region. To that end, a preliminary entomological survey was conducted in Somali Region, Eastern Ethiopia in 2018 and 2019. Four sites (Jigjiga, Degehabur, Kebrihedar and Godey) were sampled to determine the spread and distribution and relative abundance of *Aedes aegypti* from October to December 2018 and mosquito were also collected from August to December 2019 in Kebredihar following the surveillance of *An. stephensi* in East Ethiopia. Mosquitoes were collected using CDC light traps (CDC LT), pyrethrum spray collection (PSC), and aspirator from human leg sitting outdoor during at dusk and animal shed (cow and goat shed) early in the morning. Adult *Aedes* spp were identified based on morphological characteristics using standard key. A total of 280 wild caught adult *Ae. aegypti* mosquitoes were collected from the four sites of Somali region. The study revealed that *Aedes aegypti* was found in all sites and relatively, high number of *Ae. aegypti* were collected in Kebridehar town (n=268) followed by Godey (n=6), Degehabur(n=5) and Jigjiga(n=1). Most *Ae. aegypt* were caught using PSC (n=190) followed by aspirator from human leg sitting outdoor (n=62) and least were CDC LT (n = 28). *Ae. aegypti* were collected from outdoor and indoor, in Kebridehar town more *Ae. aegypti* was caught from indoor using PSC than other collection method aspirator and CDC LTs during the collection time. No *Ae. aegypti* were detected from cow and goat shelter using aspirator. The result shows *Ae. aegypti* were found close to human due to the presence of breeding habitat like cemented water reservoir local name called Birka inside human dwelling. The study provides preliminary evidence of *Ae. aegypti* in all study sites including Jigjiga, Degahabour, Kebridehar and Godey towns. These findings are of importance in the planning and implementation of vector control strategy in the region.

1062

NEEDS ASSESSMENT FOR EMERGING VECTOR-BORNE DISEASE THREATS IN THE SOUTHEASTERN REGION OF THE UNITED STATES

Danielle Johnson[1], Melissa Nolan[1], Kaci McCoy[2], Rhoel Dinglasan[2]

[1]*University of South Carolina Epidemiology and Biostatistics, Columbia, SC, United States,* [2]*CDC Southeastern Center of Excellence in Vector Borne Diseases at the University of Florida Emerging Pathogens Institute, Department of Infectious Diseases & Immunology, Gainesville, FL, United States*

Diseases transmitted by insect vectors – vector-borne diseases (VBDs) – are a major public health problem worldwide. Recently, VBDs have expanded into new environments, extended in seasonal transmission duration, and remerged in areas previously eliminated. In the US, 80% of vector control agencies lack critical resources for effectively mitigating VBD transmission locally; however, the precise resource and capacity needs are not clear. To evaluate the key opportunities for improvement, we constructed a needs assessment survey distributed to local and state vector control agencies in 13 southeastern states (SC, NC, TN, GA, FL, AL, MS, WV, VA, KY, LA, AK and MO). A high response rate was received from electronic and hardcopy mailed surveys sent to over 400 unique entities. The majority of collaborating vector control agencies serviced moderate size metropolitan areas (<1 Million population), were not affiliated with the local public health agency, did not perform tick surveillance, only performed mosquito surveillance in the extended summer season, and relied on outside agencies for pathogen testing. In contrast, the majority perform regular

mosquito insecticide resistance monitoring and utilized GIS services for mapping and planning surveillance activities. The predominant need identified by southeastern vector control agencies was personnel, basic operating resources, training, and funding. The results of our survey are being shared with state-level public health officials and legislature to raise attention as to the critical needs available training opportunities to support local vector control agencies moving forward.

1063

CONTINUED EVALUATION OF MOSQUITO EXCRETA/FECES AS AN ALTERNATIVE TOOL FOR MOLECULAR XENOMONITORING: RESULTS FROM CENTRE REGION, CAMEROON

Nils Pilotte[1], Joseph Pryce[2], Michael F. Zulch[3], Benjamin D. Menze[2], Charles S. Wondji[2], Lisa J. Reimer[2], Steven A. Williams[1]

[1]*Smith College, Northampton, MA, United States,* [2]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [3]*Boston University, Boston, MA, United States*

Previous research has demonstrated proof-of-concept for mosquito excreta/feces (E/F) testing by PCR to serve as an alternative xenomonitoring approach for diseases such as lymphatic filariasis and malaria. Similarly, a combination of field studies and laboratory-based investigations have demonstrated the capacity for mosquito E/F sampling to facilitate the detection of pathogens not vectored by mosquitoes, including *Trypanosoma brucei brucei*, and *Mansonella perstans*. However, despite a growing understanding of the capabilities and limitations of E/F testing by PCR, defining the positivity/negativity relationships that exist between testing mosquito carcasses and their corresponding E/F samples remains a challenge. In an attempt to better understand these relationships, we tested mosquito carcasses and paired E/F samples from 360 mosquitoes collected in the Centre Region of Cameroon. Following species identification and determination of gravid status, all carcasses underwent testing for the presence of *Plasmodium falciparum*, *Mansonella perstans*, *Wuchereria bancrofti* and *Loa loa*. Corresponding E/F samples were tested for the same set of pathogens. Testing for the presence of *P. falciparum* resulted in significant levels of positivity using both sample types. Interestingly, despite a complete absence of positivity for *W. bancrofti* as determined by the testing of individual mosquito carcasses, a minority of pooled E/F samples did show detection of *W. bancrofti* signal, with positivity verified by the use of multiple, independent molecular assays. Testing for the presence of *M. perstans* also demonstrated greater positivity in E/F samples than in corresponding mosquito carcasses. In contrast, while 1.4% of carcass samples tested positive for the presence of *L. loa*, all E/F samples produced *L. loa*-negative results. Taken together, these findings provide further evidence of the possible utility of E/F testing and highlight the need for additional research aimed at more fully understanding the mosquito carcass-E/F relationship.

1064

SPATIAL DISTRIBUTION AND DISPERSION OF MALARIA VECTORS ACROSS LOCAL MICRO-HABITATS IN THE PERUVIAN AMAZON

Edgar Manrique[1], Manuela Herrera-Varela[1], Marlon Saavedra[1], Samantha Solis[1], Joseph M. Vinetz[2], Gabriel Carrasco-Escobar[3], Jan E. Conn[4]

[1]*Laboratorio ICEMR-Amazonia - Universidad Peruana Cayetano Heredia, Lima, Peru,* [2]*Section of Infectious Diseases, Yale University School of Medicine, New Haven, CT, United States,* [3]*Division of infectious diseases, Medicine School, University of California San Diego, San Diego, CA, United States,* [4]*Wadsworth Center, New York State Department of Health; Department of Biomedical Sciences, School of Public Health, State University of New York at Albany, Albany, NY, United States*

Malaria vector control strategies rely on adequate sampling and estimation of mosquito distribution and abundance. In the Peruvian Amazon, this has been restricted to collecting mosquitoes around and inside households leaving most of the landscape (mainly forested areas) unsampled. Sampling strategies that focus on the representation of ecological strata are ideal for determining vector population dynamics. We sought to determine the abundance and distribution of *Nyssorhynchus darlingi* in a community of the Peruvian Amazon by sampling spatially representative grids of gradients of environmental and physical determinants. The study area was divided into 123 hexagonal grids of 1.6 ha and classified into five clusters using information derived from satellite imagery. The five landscape clusters are Forest, Deforested patches, Crops, Flooded areas, and Households. We conducted hourly collections using Human Landing Catch for 12 hours (18:00-06:00 h) at 20 randomized sampling points proportionally distributed among the clusters. A generalized linear model assuming a Poisson distribution was fitted to determine the relationship between *Ny. darlingi* abundance and cluster type, adjusted by anopheline diversity (Simpsom Index), distance to households (meters), temperature (°C), and relative humidity. After two sampling months, 3,069 female anophelines were collected, 1,348 of which were *Ny. darlingi* and the others correspond to seven anopheline species. The *Ny. darlingi* ratio of total anophelines captured for Households is 0.96 (se = 0.08), while the ratio for Forest is 0.29 (se = 0.17). The ratio for Deforested patches, Crops and Flooded areas was 0.17 (se = 0.11), 0.47 (se = 0.18), and 0.79 (se = 0.23) respectively. When compared with Forest, *Ny. darlingi* was 96% higher in Household, and 13%, 27% and 42% lower in Deforested patches, Crops and Flooded areas respectively ($p < 0.01$). This study demonstrates the wide range of *Ny. darlingi* despite its great preference for sites with anthropogenic activity. These findings, in addition to the mainly exophagic behavior of *Ny. darlingi*, provide evidence to reorient vector control measures.

1065

COEXISTENCE OF *AEDES ALBOPICTUS* AND *AEDES AEGYPTI* IN A DENGUE ENDEMIC AREA OF COSTA RICA WITH A RECENT INTRODUCTION OF *AE. ALBOPICTUS*

Adriana Troyo, Tannia Gomez-Quesada, Rolando D. Moreira-Soto, Luis M. Romero-Vega, Eugenia Corrales-Aguilar, Juan C. Cambronero-Heinrichs, Olger Calderon-Arguedas

Universidad de Costa Rica, San Jose, Costa Rica

Costa Rica is endemic for dengue; although *Aedes aegypti* is the main vector, *Aedes albopictus* has been rapidly expanding its geographical distribution. The aim of this preliminary study was to describe characteristics of the *Ae. albopictus* infestation and its coexistence with *Ae. aegypti* in Barranca, a district with dengue virus (DENV) transmission where *Ae. albopictus* was recently introduced. We carried out entomological surveys during wet (2018) and dry (2019) seasons, where we characterized larval habitats and collected adult mosquitoes using gravid and CDC (CO_2) traps in a sample of properties (houses, parks, etc.). The presence of human blood was evaluated in a subset of female *Aedes,* as well as RNA of DENV in pools of adults and larvae. In the wet season, 95% of 207 adult *Aedes* collected were *Ae. albopictus*, while only 33% (of 40) in the dry season (all others were *Ae. aegypti*). Human blood was present in both *Ae. aegypti* (20/34) and *Ae. albopictus* (25/45) females. Overall 33% (39/120) and 8% (8/98) of properties had *Aedes*-positive breeding sites in the wet and dry seasons, respectively. Breteau indices for *Aedes* were higher in the wet (43 for *Ae. aegypti*; 28 for *Ae. albopictus*) than in the dry (8 for *Ae. aegypti;* 1 for *Ae. albopictus*) seasons. Both species coexisted in 39% (24/61) of larval habitats in the wet season, but only one breeding site with *Ae. albopictus* (mixed) was recorded in the dry season. RNA of DENV was detected in pools of females, males, and larvae of both *Ae. aegypti* and *Ae. albopictus*. The main types of larval habitats were outdoor cans and plastic containers, tires, plastic tarps, and others (flower pots, drums, toilets, etc.), which fill with rainwater. Results show that both *Ae. aegypti* and *Ae. albopictus* coexist in Barranca, where they share man-made breeding sites and bite humans. Although *Ae. aegypti* is considered the vector of DENV, the virus is also present in *Ae. albopictus*. It is early to determine how this coexistence may affect dengue

epidemiology and if *Ae. aegypti* will be displaced in this area, considering the marked differences in seasonality, a mixed landscape, possible interspecific mating, and potential niche shifts.

1066

MOSQUITO DIVERSITY AND BLOODFEEDING PATTERNS IN TWO ARBOVIRUS ENDEMIC TRANSMISSION AREAS IN COSTA RICA

Luis M. Romero[1], Adriana Troyo[1], Martha Piche[1], Claudio Soto[1], Daniel Barrantes[1], Luis Chaves[2], Eugenia Corrales[1]

[1]*Centro de Investigación en Enfermedades Tropicales, San Jose, Costa Rica,* [2]*Instituto Costarricense de Investigación y Enseñanza en Nutrición y Salud, Tres Rios, Costa Rica*

Mosquito bloodmeals are a key factor for understanding arboviruses dynamics. Different landscapes influence the feeding patterns of mosquito species. The aim of this study was to perform a baseline study on mosquito bloodmeal preferences in Cuajiniquil and Talamanca, two endemic areas for arbovirus transmission in Costa Rica, We captured bloodfed females using CDC Traps for gravid females. Traps were baited with a hay infusion on a 1:10 5 proportion with water. Traps were set from 18:00 to 6:00 hours in 3 different areas, peridomiciliary, pen and forest . Altogether, our sampling effort was of 96 trap-nights. A total of 324 female mosquitoes comprising 16 different species were captured. Overall, 181 mosquitoes had visible blood in their abdomens. Species richness was assessed by rarefraction curves, and Chao2 Index was used to estimate β- Diversity. To identify the blood source, a region of the Cytochrome Oxidase subunit I gene of verebrates was amplified by PCR, sequenced, and compared to published sequences using the BLAST tool. The most common mosquito species captured was *Culex quinquefasciatus* followed by *Cx. corniger*. For the rarefraction curve, the asymptote was reached in Talamanca, but not in Cuajiniquil. Chao2 index was 22.32 (SD= 16.90) for Cuajiniquil and 12.99 (SD= 3.72) for Talamanca. Chicken (54.5%) was the most common feeding source detected followed by human (20.0%). Dogs, cats, cows, doves and iguanas were also detected. Even though, chickens are not an important enzootic host for arboviruses that have been reported in these areas, their presence may be considered zooprophilactic, as chickens attract the most common mosquito species and decrease contact human-vector contact . Nevertheless, for thoroughly understanding arboviral dynamics in these endemic areas, viral detection is needed and in progress.

1067

VECTOR COMPETENCE OF TWO *AEDES AEGYPTI* MOSQUITO POPULATIONS FROM THE U.S BORDER REGION TO DENGUE VIRUS

Pedro M. Palermo, Jeanette Orbegozo, Douglas M. Watts

University of Texas at El Paso, El Paso, TX, United States

Dengue is considered to be the most important mosquito-borne viral diseases among humans. Dengue viruses are endemic in tropical and subtropical regions inhabited by the vectors *Aedes aegypti*, and *Ae. albopictus*. Several dengue outbreaks have been reported in the Rio Grande Valley of southeast Texas and northern Mexico during the past 30 years. Even though *Ae. aegypti* inhabits the entire southern United States (US) border region; little is known about the vector competence of this mosquito for dengue viruses (DENV) in this region. In this study, we compared the vector competence of *Ae. aegypti* mosquito from El Paso and Rio Grande Valley of Texas for DENV serotype 2 genotypes. *Ae. Aegypti* mosquitoes from both urban border communities were susceptible to DENV infection with infectivity rates ranging from 65% to 88% at 14 days post-exposure. In addition, the viral infectious dose and the time post-exposure variables were associated with an increase in infectivity rates. Dissemination and transmission rates are being determined to provide the information needed to assess the risk of DENV transmission in this US Texas-Mexico border region.

1068

PRELIMINARY INVESTIGATIONS INTO THE APPLICATION OF DNA-LOADED PROTEIN CRYSTALS FOR MOSQUITO MARK-RELEASE-RECAPTURE STUDIES

Daniel A. Hartman, Lyndsey Gray, Julius Stuart, Christine Hirt, Therese Kondash, Alec Jones, Christopher Snow, Rebekah C. Kading

Colorado State University, Fort Collins, CO, United States

The application of Mark-Release-Recapture (MRR) methods to mosquito populations has proven indispensable to the inference of dispersal, survival, and population sizes of mosquitoes under field conditions. Currently available technologies for marking mosquitoes are quite limited in terms of information content and efficacy, with limited numbers of unique markers and negative effects on mosquito biology. To overcome both challenges, we have engineered and laboratory-tested a new class of biomarkers, in which information-rich synthetic DNA is protected within tough, crosslinked protein crystals. The application of DNA barcodes to MRR opens the possibility to deploy many markers simultaneously, representing several spatial locations and time intervals. We have demonstrated that these crystals are ingestible by larval mosquitoes and persist transtadially to adults. DNA barcode sequences are recoverable by qPCR with an assay that is specific to the synthetic barcode. Barcode sequences are detectable in pools of up to 50 mosquitoes, enabling the technology to be incorporated efficiently into established surveillance programs. Adult mosquitoes reared on microcrystals showed no significant reduction in survival compared to non-crystal-exposed mosquitoes, supporting adequate recapture rates for field investigations. These DNA-laden protein microcrystals have the potential to radically increase the feasible information content compared to conventional mark-release recapture particles.

1070

MOLECULAR-BASED APPROACHES REVEALED NEW *ANOPHELES* CRYPTIC SPECIES INVOLVED IN HUMAN MALARIA TRANSMISSION IN WESTERN KENYA

Daibin Zhong[1], Elizabeth Hemming-Schroeder[1], Xiaoming Wang[1], Solomon Kibret[1], Guofa Zhou[1], Harrysone Atieli[2], John Githure[2], Yaw A. Afrane[3], Andrew K. Githeko[2], Guiyun Yan[1]

[1]*University of California Irvine, Irvine, CA, United States,* [2]*Kenya Medical Research Institute, Kisumu, Kenya,* [3]*University of Ghana, Accra, Ghana*

A thorough understanding of malaria vector species composition and their behavioral characteristics is crucial to devise effective and efficient vector control interventions to reduce malaria transmission. It has been well documented that malaria interventions in the past decade have induced major changes in malaria vector species composition from endophilic *Anopheles gambiae s.s.* to exophilic *An. arabensis* in Africa. However, less is known about malaria vector species community structure changes and whether new cryptic sibling species may emerge and play more important role in malaria transmission. This study examined the composition, distribution of malaria vectors, with a particular focus on malaria transmission potential of new, uncharacterized *Anopheles* species and genotypes in Western Kenya. *Anopheles* mosquitoes were collected from lowland and highland settings and molecular identity of species were determined using multiplex-PCR or sequencing of ribosomal DNA internal transcribed spacer region 2 (ITS2) and mitochondrial DNA cytochrome oxidase subunit 1(COX1) genes. Phylogenetic analysis revealed 21 sequence groups or molecularly identified species. Unusually high rates of *Plasmodium* sporozoite infections were detected in *An. funestus s.s.*, *An. gambiae s.s.* and eight cryptic rare species, including two previously unreported cryptic species. *Plasmodium falciparum, P. malariae* and *P. ovalae* sporozoite infections were identified. Higher *Anopheles* species diversity was found in the highlands (11 *Anopheles* sp.), as compared to the lowlands (3 *Anopheles* sp.). This study, for the first time, reported eight

rare *Anopheles* species with *Plasmodium* sporozoite infections in Western Kenya. The findings of this study call for the need to pay attention on the biology of these rare malaria vector species in vector surveillance.

1071

A MECHANISTIC MODEL FOR HOW TEMPERATURE AFFECTS TRANSMISSION OF LYMPHATIC FILARIASIS BY *AEDES POLYNESIENSIS*

Sindiso Nyathi, Marta Shocket, Erin Mordecai

Stanford University, Stanford, CA, United States

Lymphatic Filariasis is a neglected tropical disease caused by nematode parasites (primarily *Wucheria bancrofti*) and transmitted by a range of mosquito vectors. The global burden of LF is 120 million people, with about 886 million at risk, primarily in tropical areas. The main vector of LF in Pacific Island nations is *Aedes polynesiensis.* We fit thermal responses to previously published data and built a mechanistic model that quantifies how these non-linear, temperature-dependent mosquito and parasite traits affect the basic reproductive ratio, R_0. This model can estimate the influence of changing climate and seasonality on the geographic distribution, incidence and burden of LF. We collected laboratory derived, temperature-dependent trait data from the literature for *Ae. polynesiensis* and *W. bancrofti* traits required by a standard R_0 model. We used a Bayesian approach to fit thermal responses to these data. This approach allowed us to incorporate relevant trait data from other mosquito species as priors and estimate uncertainty in the resulting thermal response curves. We used these thermal response curves in the R_0 model to estimate the influence of temperature on transmission of *W. bancrofti* by *Ae. polynesiensis.* We found appropriate data for *Ae. polynesiensis* egg viability, adult lifespan, larval survival, and larval development rate, as well as *W. bancrofti* parasite development rate. Preliminary results indicate that the thermal response curves of these traits are unimodal, peaking between 24ºC and 33ºC. Data for *Ae. polynesiensis* biting rate and fecundity, and *W. bancrofti* vector competence were not available, and were substituted with data from similar species. The model predicts that transmission of LF peaks at 27ºC and is limited at temperatures below 15ºC and above 34ºC. Further, LF transmission peaks at intermediate temperatures, matching results in other mosquito-borne diseases. This result suggests that changes in climate will result in shifts in the transmission of LF, with cooler regions having increased risk of LF, and warmer regions (currently at highest risk) experiencing a decreased risk of LF.

1072

A COMPARATIVE ANALYSIS OF DENGUE, CHIKUNGUNYA, AND ZIKA MANIFESTATIONS IN A PEDIATRIC COHORT OVER 17 YEARS

Fausto A. Bustos Carrillo[1], Kendall Lee[1], Sergio Ojeda[2], Nery Sanchez[2], Miguel Plazaola[2], Damaris Collado[2], Tatiana Miranda[2], Saira Saborio[2], Brenda Lopez[2], Jairo C. Monterrey[2], Leah Katzelnick[1], Douglas Elizondo[2], Sonia Arguello[2], Amy Brandt[3], Lora Campredon[3], Krista Latta[3], Aubree Gordon[3], Angel Balmaseda[4], Guillermina Kuan[5], Eva Harris[1]

[1]*Division of Infectious Diseases & Vaccinology, School of Public Health, University of California, Berkeley, Berkeley, CA, United States,* [2]*Sustainable Sciences Institute, Managua, Nicaragua,* [3]*Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, Ann Arbor, MI, United States,* [4]*Laboratorio Nacional de Virología, Centro Nacional de Diagnóstico y Referencia, Ministry of Health, Managua, Nicaragua,* [5]*Centro de Salud Sócrates Flores Vivas, Ministry of Health, Managua, Nicaragua*

Dengue, chikungunya, and Zika are arboviral diseases with overlapping clinical features, making differential diagnosis difficult in the absence of laboratory testing. Small sample sizes, historical characterization of these diseases in adults, and incomplete case ascertainment (especially for Zika) have compounded this problem. Here, we contrast the pediatric clinical features of these 3 diseases over 17 years in Managua, Nicaragua. Clinical and demographic data from laboratory-confirmed cases aged 2-17 years in the Pediatric Dengue Cohort Study (PDCS) were extracted and analyzed. Health center and hospital data were included, as were data from a parallel flu cohort when data were available. Generalized additive, linear, and mixed-effects models, logic regression, classification trees and hierarchical clustering were used to analyze the data. Clinical features of 1,035 dengue, 539 chikungunya, and 556 Zika cases were assessed. We describe, for the first time, 43 RT-PCR-confirmed cases of dengue without fever or a history of fever. The prevalence of many clinical features (signs, symptoms, and complete blood count) differed across pediatric age, particularly for fever, rash, and arthralgia. Peri-articular edema was almost never observed in dengue and Zika cases, and papular rash was never observed in chikungunya cases. The temporal dynamics of fever, rash, and arthralgia prevalence were the most dissimilar across the diseases, with chikungunya cases displaying increases in generalized erythematous rash on day 6 and 7 of illness. The average temperature of dengue and chikungunya cases were similar across the first 7 days of illness, while temperatures of Zika cases were noticeably milder over most of the acute illness period. The classic biphasic fever of dengue was only observed for 8% of uncomplicated dengue cases, although this percentage is reported to be higher for cases with severe dengue disease. Overall, these results substantially update the clinical epidemiology of pediatric arboviral diseases and point to differences in fever, rash, and arthralgia that could be used to better inform arboviral differential diagnosis.

1073

THE USE OF THE GOOGLE EARTH ENGINE AND THE EWARS MODEL TO TRACK DENGUE SURVEILLANCE IN LIPA CITY, PHILIPPINES

Tiago Canelas, Edward Thomsen

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Dengue is the fastest-spreading mosquito-borne disease worldwide transmitted by the *Aedes (Stegomyia) aegypti*. The ability to detect early outbreaks is key to have an effective response. The early-warning response system (EWARS) is a tool by the WHO running in R software that provides early-warnings of dengue outbreaks. EWARS uses the outbreak indicator (i.e. dengue cases) alongside alarm indicators (i.e. rainfall) to prospectively predict the outbreak. Dengue outbreaks have been associated with some meteorological variables as temperature, precipitation, or humidity and to run the tool there must be at least one alarm indicator for a minimum of three years. In order to collect these variables at the sub-district level an extensive and expensive network of meteorological stations is needed. Most of the dengue burden happens in areas where such network is unfeasible and one approach to overcome this lack network is using remote sensing images to collect the data. The workflow of using satellites images to extract this information might be tedious, laborious, time-consuming and where internet speed not very fast almost impossible. Google Earth Engine (GEE) is a computing platform that allows to run geospatial analysis on Google's infrastructure. With extensive imagery datasets, the user can introduce the variables of interest and the sub-district boundaries and calculate the alarm indicator for multiple years at once. We used this approach to test the feasibility of collection, mean temperature, total precipitation, and mean relative humidity for the 72 barangays (administrative unit) from 2012 to 2019. Despite the enormous amount of data, the processes were extremely fast and accurate for the purpose. These results open new possibilities to collect data in a more efficient and straightforward way. Health officers using EWARS should integrate GEE in their data collection. One future step will be including the data extraction into the EWARS tool, for this, an R package "rgee" allows the communication between both, eventually, enabling everything to run in a single platform.

1074

TESTING FOR ANTI-DENGUE VIRUS AND ANTI-ZIKA VIRUS IGG ON A PLASMONIC GOLD PLATFORM AND COMPARISON WITH NEUTRALIZATION TESTING

Alejandra Rojas[1], Muktha S. Natrajan[2], Jenna Weber[3], Fátima Cardozo[1], Sanny López[1], César Cantero[1], Cynthia Bernal[1], Yvalena Guillén[1], Laura Mendoza[1], Melvina Páez[1], Benjamin A. Pinsky[3], **Jesse J. Waggoner**[2]

[1]*Instituto de Investigaciones en Ciencias de la Salud, Universidad Nacional de Asunción, San Lorenzo, Paraguay,* [2]*Emory University, Atlanta, GA, United States,* [3]*Stanford University, Palo Alto, CA, United States*

Antibody cross-reactivity confounds testing for dengue virus (DENV) and Zika virus (ZIKV). Recently, a multiplex serological assay on a plasmonic gold platform (termed the pGOLD assay) demonstrated sensitive detection and differentiation of IgG responses to DENV (whole-virus antigen) and ZIKV [non-structural protein 1 (NS1) antigen]. In this study, we compared anti-DENV and anti-ZIKV IgG detection in the pGOLD assay to focus reduction neutralization testing ($FRNT_{50}$) among patients from metro Asunción, Paraguay, which experiences annual DENV outbreaks but reported few autochthonous ZIKV infections. Acute-phase sera (≤8 days post symptom onset) were tested from 77 patients who presented with a suspected arboviral illness from January-May 2018. Thirty-nine patients (50.6%) had acute dengue, diagnosed by rRT-PCR or an immunochromatographic NS1 assay. No acute ZIKV infections were detected by rRT-PCR. Sixty-five of 77 patients (84.4%) had anti-DENV neutralizing antibodies. Only 3 patients had low-titer ZIKV neutralizing antibodies (1:55-1:80) and all had high corresponding DENV neutralizing titers > 1:4,000. Anti-DENV IgG detection in the pGOLD assay demonstrated good agreement with $FRNT_{50}$ (kappa = 0.74), and quantitative antibody levels were highly correlated between the two methods ($p<0.001$). Anti-ZIKV signal was detected in the pGOLD assay in 16 samples (20.8%), which likely resulted from cross-reactive antibodies as it occurred among patients with high corresponding titers of anti-DENV IgG (median maximum $FRNT_{50}$, 1:2,631). Nine patients were hospitalized for dengue. Compared to outpatients, hospitalized cases had significantly higher levels of anti-DENV antibodies measured by either method ($p<0.001$). All 6 dengue cases with anti-ZIKV IgG detected in the pGOLD assay required hospitalization, which supports the potential pathophysiologic significance of anti-NS1 antibodies, as detected in the pGOLD ZIKV assay. This study demonstrated the performance of the pGOLD assay, which is faster and simpler than FRNT. In addition, this new method may provide data for patient risk stratification in the acute setting.

1075

MOLECULAR MECHANISMS THAT REGULATE FLAVIVIRUS NS1 DISRUPTION OF INTERCELLULAR JUNCTION PROTEINS, LEADING TO ENDOTHELIAL BARRIER DYSFUNCTION AND VASCULAR LEAKAGE

Henry N. Puerta-Guardo, Scott B. Biering, Francielle Tramontini Gomes de Sousa, Jeffrey Shu, Jeffrey Li, Dustin Glasner, Eva Harris

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

Flavivirus NS1 proteins were recently shown to directly cause endothelial barrier dysfunction and vascular leakage in a tissue-dependent manner via disruption of the endothelial glycocalyx layer (EGL) lining the endothelium. Together with the EGL, the intercellular junction complex (IJC) composed of tight and adherens junction (TJ/AJ) proteins modulates vascular permeability. However, which signaling pathways contribute to flavivirus NS1 disruption of TJ/AJ barriers is still unclear. Here, we investigated the relative contribution of 5 flavivirus NS1 proteins from dengue (DENV), Zika (ZIKV), West Nile (WNV), Japanese encephalitis (JEV), and yellow fever (YFV) viruses to the expression, localization, and fate of TJ/AJ proteins in human umbilical vein (HUVEC) and brain (HBMEC) endothelial cells in relation to NS1-mediated barrier dysfunction. Six hours post-treatment, immunofluorescence assays demonstrated that DENV and ZIKV NS1 altered distribution of ZO-1, VE-cadherin, and β-catenin in HUVEC monolayers, while in HBMEC, this distribution was affected by all flavivirus NS1 proteins except YFV NS1. This pattern is consistent with NS1-induced endothelial hyperpermeability and viral disease tropism. Western blot analyses showed no significant reduction in expression of TJ/AJ proteins in both cell lines after NS1 treatment, except for ZO-1 in response to DENV NS1 in HUVEC and WNV NS1 in HBMEC. We also found that DENV NS1 induces colocalization of ZO-1 and VE-cadherin with clathrin-coated pits and phosphorylation of β-catenin (Ser45), which results in remodeling of the IJC. Specific inhibitors of GSK-3β and an upstream kinase, phosphoinositide 3-kinase, reduced NS1-induced permeability of HUVEC monolayers *in vitro* and vascular leakage in a mouse dorsal intradermal model, indicating that these players are also involved in the NS1-induced endothelial dysfunction. These findings contribute insights into the molecular mechanisms that regulate NS1 modulation of TJ/AJ proteins leading to endothelial barrier disfunction and identify potential host targets to prevent vascular leakage during severe dengue disease.

1077

RAPID POINT OF CARE DIAGNOSTIC TESTS TO DETERMINE DENGUE SEROSTATUS

Yan Zhou, Jason Zhou

Zymeron Corporation, Durham, NC, United States

Rapid Point of Care Diagnostic Tests to Determine Dengue SerostatusYan Zhou[a], Jason Zhou[a], Michael Diamond[ba] Zymeron Corporation, Research Triangle Park, NC 27709[b] Washington University School of Medicine, St. Louis, MO 63110 Abstract:

Dengue virus (DENV) is a leading cause of fever in tropical regions. DENV infections can be life threatening and difficult to manage in austere settings. The four dengue virus serotypes complicate risk management. An initial dengue exposure dramatically increases the chance of a more severe infection when exposed to a second serotype. Currently there is one FDA approved dengue vaccine Dengvaxia (CYD-TDV) with several others in the pipeline. However, dengue's multiple serotypes and the resultant complex host immunity make vaccination a safety concern, requiring pre-vaccination screening of serostatus and determination of proper vaccine strategy. Zymeron has developed one-step, rapid (less than 20 minutes), low-cost, self-contained POC diagnostic tests (RapiDENV™) for determining Dengue serostatus directly from whole blood by applying its novel nanoenhanced fluorescent lateral flow immunoassay technology. Our RapiDENV™ test overcomes drawbacks (i.e. low sensitivity) of conventional lateral flow assays while taking advantage of many attributes inherent to lateral flow as an ideal rapid POC test. Such test can be used in physicians' offices or vaccination clinics for on-the-spot determination of dengue serostatus during a patient's visit.

1078

ARBOBIOS: A BRAZILIAN COHORT OF DENGUE WARNING SIGNS PATIENTS DURING 2019 EPIDEMICS

Erika R. Manuli[1], Geovana M. Pereira[1], Felipe ten Caten[1], Mariana S. Ramundo[1], Bruno H. Milhim[2], Flavio A. Milagres[3], Angela A. Costa[4], Fernanda C. Alcântara[5], Crhistinne C. Goncalves[6], Ester C. Sabino[1], Glaucia Paranhos-Baccalà[7]

[1]*Instituto de Medicina Tropical, Sao Paulo, Brazil,* [2]*Faculdade de Medicina de Sao Jose do Rio Preto, Sao Jose do Rio Preto, Brazil,* [3]*Universidade Federal do Tocantins, Palmas, Brazil,* [4]*Serviço Especial de Saúde de Araraquara-USP, Araraquara, Brazil,* [5]*Universidade Federal de São João del-Rei, Divinopolis, Brazil,* [6]*Faculdade de Medicina da Universidade Federal do Mato Grosso do Sul, Campo Grande, Brazil,* [7]*bioMerieux, Sao Paulo, Brazil*

It is known that 3.6 billion people worldwide live in areas that place them at risk of DENV infection, 400 million overall are exposed to DENV infection. Around 2 to 5 % of infected individuals progress to Severe Dengue (SD). The mortality can be reduced to less than 1% if robust early predictor of progression to SD exists.To establish a warning signs

cohort for identification and validation of prognostic biomarkers for the severe DENV infection. During the 2019 epidemic in Brazil, a prospective longitudinal cohort of warning signs (WS) DENV patients was constitute in the following cities: Araraquara, SP; Arcos, MG; Campo Grande, MS; Nova Serrana, MG; Palmas, TO and São José do Rio Preto, SP. Two following-up visits were programmed at days 7 and 14. The presence of RNA DENV virus were done by in-brew qPCR after RNA extraction with EasyMag (bioMérieux) and serological responses were evaluate by IgM ELISA (PanBio) only for samples negative in qPCR. For this study, we used SMS data banking. A total of 1117 patients were enrolled and 48 refused to participate. 1069 were analyzed and from those 1016 were adults. The virus RNA was detected in 36.2% of the samples (387) most of them were serotype 2, and out of 682 negative for virus RNA, 444 were reactive in the serological test (65.10%), it is assumed that 77.7% (831) of the participants presented DENV infection at the time of the study. Only 1.59% (17) were not detected by both tests. The follow-up were realized in 984 and 960 patients for days 7 and 14, respectively. This is the biggest WS DENV clinically well characterized cohort in Brazil. As expected, we detected the presence of DENV virus in a low number of WS DENV patients, however IgM serological test were positive in most of negative samples for qPCR. The follow-up were concluded but each medical record should be analyzed and revised to estimate the number of SD. The next step of this study is process the samples for transcriptomic and micro RNA analyses.

1079

COMPUTATIONALLY DESIGNED STABLE DENGUE VIRUS-2 ENVELOPE DIMER SUBUNITS INDUCE EFFICIENT NEUTRALIZING ANTIBODY RESPONSES IN MICE

Devina Thiono, Stephan Kudlacek, Shaomin Tian, Ashlie Thomas, Alexander Payne, Stefan Metz, Brian Kuhlman, Aravinda de Silva

University of North Carolina, Chapel Hill, NC, United States

The four dengue virus (DENV) serotypes infect around 390 million people per year, with clinical symptoms ranging from mild disease to severe and potential fatal dengue hemorrhagic fever and shock syndrome. The current leading DENV vaccine candidates are based on a tetravalent mix of live-attenuated viruses. However, it struggles to induce balanced protection against the four serotypes mainly due to unbalanced replication of vaccine components resulting in an effective immunity to just one serotype. Protein subunit vaccines are safe and easy to formulate as balanced tetravalent vaccines because immunogenicity is not dependent on viral replication. However, recombinant DENV Envelope protein (rE) subunit vaccines have not performed particularly well in animal and human studies. This is partly because the previously tested rE subunits are mainly present as monomers at physiological conditions while most human antibodies that strongly neutralize DENVs recognize epitopes of quaternary structure that are only present on E-homodimers and higher-order structures on the viral surface. We hypothesized that rE homodimer subunits can mimic the quaternary structures of envelope protein on DENVs surface, and thus it can be a better vaccine antigen than the previously used rE-monomer. Using the structure-based computational design program Rosetta, we have designed and produced rE proteins that form stable homodimers at 37°C. Here we present results on the structural and antigenic properties of wild-type (WT) monomeric rE proteins and stabilized rE homodimer variants. The stabilized rE homodimer variants have improved binding affinity to E-dimer specific neutralizing antibodies compared to WT rE. Immunogenicity studies in mice indicate that dimeric rE-antigens show a stronger correlation between DENV2 specific IgG titers and neutralizing efficiency compared to monomeric rE antigens. These findings suggest that the oligomeric state of the dengue rE protein has a strong impact on immunogenicity and functional neutralization. Our data suggest structure-guided protein design is a promising strategy for developing dengue subunit vaccine.

1080

DENGUE CASES IN U.S. STATES AND TERRITORIES, 2019

Laura Adams, Aidsa Rivera, Gabriela Paz-Bailey, Stephen H. Waterman

CDC Dengue Branch, San Juan, PR, United States

Dengue is the leading cause of arboviral disease globally, causing an estimated 390 million infections annually. During 2019, dengue cases reached record numbers in the Americas, with over 3 million cases reported to the Pan American Health Organization (PAHO). In the United States, dengue cases from states and territories are reported to ArboNET, the national arboviral surveillance system. Here, we report dengue cases from 2019 as of April 2020, and compare trends from previous years and by reporting jurisdiction. Numbers are preliminary and will be updated in the final presentation. During 2019, 1,539 confirmed and probable dengue cases were reported to ArboNET; 51% were female, with a median age of 42 years (range 1-96). A total of 656 (43%) cases were hospitalized, 30 (2%) were categorized as severe dengue, and 4 (0.26%) fatal cases were reported. The majority (n=1,423; 92%) of cases were reported from states, of which 1,400 (98%) were travel-associated. This represents the highest number of cases reported by states since reporting began in 2010, and an increase of nearly 50% from 2016, which had the second highest number of cases reported (n = 961). The most frequently reported regions of travel included the Caribbean (38%), Americas (27%), and Asia (27%). Jurisdictions reporting the highest numbers of cases in 2019 included Florida (n = 410; 27%), California (n = 263; 17%), and New York (n = 128; 8%). A total of 116 (8%) of all cases were reported from territories and affiliated states, of which 82% were locally-acquired. Dengue cases reported in the territories were younger (mean 29; 95% CI 25-33 years) than cases reported from the states (mean 42; 95% CI 40-43 years). Record numbers of dengue cases were reported to ArboNET during 2019, most of which reported travel in the Americas and Caribbean, and more than half of which were concentrated in 3 states.

1082

CHARACTERIZATION OF SEROLOGICAL RESPONSE TO DENGUE AND ZIKA VIRUSES IN PREGNANT WOMEN DURING THE ZIKA OUTBREAK IN BRAZIL

Kaitlin Driesse[1], Wen-Yang Tsai[1], Carlos Brites[2], Celia Pedroso[2], Wei-Kung Wang[1]

[1]*John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, HI, United States,* [2]*LAPI-Laboratório de Pesquisa em Infectologia-School of Medicine, Federal University of Bahia, Salvador, Brazil*

The outbreak of Zika virus (ZIKV) and associated congenital Zika syndrome in dengue virus (DENV)-endemic regions raised important questions about the effects of ZIKV infection on DENV immunity and disease outcome and vice versa, which further highlighted the need of serological tests that can discriminate different DENV and ZIKV infections. Previously we developed combined DENV and ZIKV non-structural protein 1 (NS1) enzyme-linked immunosorbent assays (ELISAs) to overcome the cross-reactivity of traditional envelope protein-based serological tests, as reported previously. In this study, we used this assay to screen 138 serum samples collected from asymptomatic parturient women during the ZIKV outbreak in Salvador, Brazil and identified four serostatus groups. We further characterized the antibody responses in 32 participants including primary DENV (pDENV), secondary DENV (sDENV), primary ZIKV (pZIKV), and ZIKV with previous DENV (ZIKVwprDENV) infections by different ELISAs and neutralization tests. Using depletion experiment with inactivated ZIKV and urea test, we determined the IgG avidity to DENV. Using depletion experiment with inactivated DENV and endpoint ELISA titers, we determined the ZIKV type-specific and cross-reactive antibody and found pZIKV had higher proportion of ZIKV type-specific antibody (82%) than ZIKVwprDENV (10-16%). These results were supported by microneutralization test. Our findings suggest that combined DENV and ZIKV NS1 IgG ELISAs plus depletion experiment can delineate past and

present DENV and ZIKV infections and are a potential tool to further our understanding of the pathogenesis and epidemiology of DENV and ZIKV in endemic regions.

1083

UBIQUITIN-CONJUGATING ENZYME 'UBE2J1' PROMOTES THE DEVELOPMENT OF DENGUE HEMORRHAGIC FEVER

Kulachanya Suwanwongse

Lincoln medical center, New York, NY, United States

Dengue infection is a common vector-borne disease, causing broad ranges of clinical presentation, from asymptotic to dengue shock syndrome. Dengue hemorrhagic fever (DHF) is a severe form of dengue infection, but its pathogenesis is unclear. We conducted a bioinformatics analysis of differentially expressed genes (DEGs) in patients with dengue infection with and without DHF to identify the key candidate genes underlying DHF pathogenesis. The gene expression profiling datasets (GSE51808) were obtained from the Gene Expression Omnibus database and analyzed by GEO2R platform. The t-test was done to compare DEGs from peripheral blood from patients with dengue infection with and without DHF. The Database for Annotation, Visualization and Integrated Discovery (DAVID) was implemented to extract biological features and processes of DEGs via gene ontology (GO) analysis and formulate the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. A Systemic search was performed to evaluate each DEGs functions and its possible association to DHF. A total of 58 DEGs were obtained. 47 genes were found to be up-regulated in DHF group. We proposed UBE2J1 is responsible for the development of DHF because of 1. UBE2J1 is needed for dengue infection. 2. Previous study confirmed a positive correlation between UBE2J1 expression and dengue virus infectivity. 3. We found an up-regulated UBE2J1 expression in DHF group. 4. GO analysis showed DHF up-regulated genes were enriched in negative regulation of retrograde protein transport positive regulation which related to UBE2J1 role. 5. KEGG analysis of DEGs formulated protein processing pathway in endoplasmic reticulum which can be explained by UBE2J1.In conclusion, UBE2J1 overexpression may lead to DHF and drugs modifying UBE2J1 expression might help in DHF treatment and prevention.

1085

ACCEPTABILITY OF DENGUE VACCINE AMONG PARTICIPANTS OF A COMMUNITY-BASED COHORT STUDY FOR ARBOVIRAL DISEASES IN PONCE, PUERTO RICO

Chelsea Grace Major[1], Liliana Sánchez-González[1], Laura E. Adams[1], Kyle Ryff[1], Dania M. Rodriguez[1], Olga D. Lorenzi[1], Stephen H. Waterman[1], Vanessa Rivera-Amill[2], Gabriela Paz-Bailey[1]

[1]*Centers for Disease Control and Prevention, San Juan, PR, United States,* [2]*Puerto Rico Health Sciences University and Saint Luke's Episcopal Hospital Consortium, Ponce, PR, United States*

In May 2019, Dengvaxia® was the first dengue vaccine approved in the USA for children aged 9–16 years with previous lab-confirmed dengue virus infection and living in endemic areas. Puerto Rico (PR) has endemic dengue transmission with widespread outbreaks every 3–5 years, making residents important candidates for an approved vaccine and vaccine trials. To assess dengue vaccine acceptability and potential implementation barriers, we used interviews from the Communities Organized to Prevent Arboviruses (COPA) cohort study in Ponce, PR. Questions on dengue vaccine acceptability, cost, and other characteristics were included in 2019–2020 annual follow-up interviews for adult participants aged 21–52 years, and data collection is ongoing. We described response data and used chi-square tests to identify factors associated with dengue vaccine acceptability. Among 1,173 participants with available responses, 73% were willing to be vaccinated with an approved dengue vaccine if free of cost, 59% would pay $10, 37% would pay $20, and 12% would pay $50 per dose. Responses were similar for parents of minors (n = 732) with 75% willing to vaccinate their children if free. Of 342 participants who would not or were unsure if they or their children would be vaccinated for dengue if free, the most common reason was concern for side effects (38%) followed by not believing in vaccination (17%). Among proposed features of a dengue vaccine, 62% of the 1,173 participants chose high level of protection as most important and 21% chose minimal side effects. Overall, males ($P = 0.0142$) and participants vaccinated for influenza in the past year ($P < 0.0001$) were more likely to be willing to be vaccinated for dengue if offered for free or $10 per dose; there were no significant differences by education level, income, medical insurance status, or prior arbovirus diagnosis. Our results suggest that dengue vaccine acceptability is high in the COPA cohort, but cost as well as safety concerns and general vaccine distrust may be important barriers. This information will be useful in informing dengue vaccine roll-out plans, educational campaigns, and suitability for vaccine trials in PR.

1086

AN EARLY AND DIFFERENTIAL SEROLOGICAL DIAGNOSTIC TEST FOR ZIKA - IN THE CENTER OF AN ARBOVIRUS CLOUD IN BRAZIL

Stella Garcia Colombarolli[1], Eneida Santos de Oliveira[1], Izabella Cristina Andrade[1], Camila Sales Nascimento[1], Rafael Ramiro de Assis[2], Carlos Eduardo Calzavara-Silva[1]

[1]*Rene Rachou Institute, Belo Horizonte, Brazil,* [2]*University of California, Irvine, CA, United States*

The Zika vírus is an arbovirus, member of the *Flaviviridae* family ZIKV infection is related to severe neurological complications in newborns, such as microcephaly, and Guillan-Barret Syndrome, in adults. In South America, especially in Brazil, other arboviruses co-circulate, such as DENV, YFV and CHIKV, causing a major public health problem, since the diseases caused by them initially show very similar symptoms, but different outcomes, and may present severe conditions that also require different clinical management and hospital and public assistance. Because of these common clinical signs, a differential, specific and sensitive diagnostic test is necessary to identify these infections early. Thus, this study aims to identify specific ZIKV epitopes, which can compose a point of care test, capable of being used at the hospital bed, in the poor countries where Zika is still a serious public health problem. Therefore, the following methodology is: 1- Using bioninformatics as a tool, ZIKV, DENV and YFV epitopes were predicted for B cells, based on the sequences available on the Virus Variation - NCBI platform, 2- ZIKV-specific antigenic peptides were selected, designed and synthesized, 3- The peptides were tested in protein microarrays to evaluate the specific recognition capacity in more than 250 samples of human sera, characterized in 4 different groups: I - positive for zika, II - positive for dengue, III - positive for yellow fever, IV - negative for the tested arboviruses. Among 40 peptides tested, one peptide - CapZIKV - from the viral capsid of ZIKV, showed excellent results during the microarray evaluation, being able to distinguish in both IgM and IgG infections caused by ZIKV, infections by DENV, YFV and the group of healthy patients. The CapZIKV peptide, when comparing the positive groups for zika and dengue, showed a remarkable result of significance, with the ability to distinguish groups with a specificity of 88.37 and sensitivity of 100 per cent. These promising results should be published and new serological tests are being carried out, this time using an ELISA, for the setting up of a point of care.

1087

IDENTIFICATION OF JAPANESE ENCEPHALITIS VIRUS GENOTYPE V AND OTHER MOSQUITO-BORNE VIRUSES IN CAMP HUMPHREYS, REPUBLIC OF KOREA, USING METAGENOMIC ANALYSIS

Mark A. Sanborn[1], Heung-Chul Kim[2], Kathryn M. Wuertz[1], Yu Yang[1], Tao Li[1], Simon D. Pollett[1], Richard G. Jarman[1], Irina Maljkovic Berry[1], Terry A. Klein[1], **Jun Hang**[1]

[1]*Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [2]*Force Health Protection and Preventive Medicine, MEDDAC-Korea/65th Medical Brigade, APO AP, MD, United States*

Recent outbreaks of emerging and re-emerging viruses such as Zika, West Nile virus and Japanese Encephalitis virus (JEV) have shown that timely detection of novel arboviruses with epidemic potential is essential to mitigate the risk to human health. There have been rising concerns that an emergent JEV genotype (genotype V, G-V) is circulating in East Asia, against which the current FDA-approved JEV vaccine may not be efficacious. To enhance understandings of arboviruses that are circulating in East Asia, Next-Gen sequencing was conducted on 276 pools of *Culex tritaeniorhynchus* and *Culex bitaeniorhynchus* mosquitoes (7,204 specimens) collected at Camp Humphreys, Republic of Korea (ROK), from May - Sept 2018. Metagenomic analysis revealed a highly abundant and diverse virome in these mosquito populations, resulting in the identification of over 20 novel and previously uncharacterized viral genomes. Two complete JEV G-V genome sequences were obtained from separate mosquito pools, indicating that JEV G-V may be circulating in the Pyeongtaek area near Seoul, ROK. Retrospective sample and sequence analyses showed that JEV G-V was also present in 2016 mosquito pools collected in Seoul. Further analysis demonstrated that both the 2016 and 2018 JEV G-V genomes share a high degree of similarity to the Chinese JEV G-V strain XZ0934 (JF915894) found in mosquitoes collected in 2009, and a clinical CSF isolate collected in ROK in 2015 (MK541529.1), with nucleotide identities of 97% and 99% to the two strains, respectively. This study highlights the critical need for continued surveillance of mosquito populations as a means of detecting and identifying emerging and re-emerging arboviruses of public health relevance. Importantly, our results emphasize recent concerns that there may be a possible shift in the circulating JEV genotype in East Asia and highlights the critical need for a vaccine that is proven to be efficacious against this potentially re-emergent virus.

1090

THE EFFECTS OF A ZIKA VIRUS INFECTION ON CAMP RESPONSIVE ELEMENT BINDING PROTEIN 3 LIKE 1 NEUROPROTECTIVE PATHWAY

Joaris Soto-Hernández

Ponce Research Institute-Ponce Health Sciences University, Ponce, PR, United States

Zika Virus (ZIKV) can act as a teratogenic agent and cause congenital zika syndrome. ZIKV can reduce the ability of human neuron progenitor cells and other nerve cells to induce self-repair and cell survival mechanisms. Neuroprotective pathway known as cAMP responsive element binding protein 3-like 1 (CREB3L1) regulates the transcription of activity-regulated inhibitor of death (AID) gene expression. This neuroprotective response is known to decrease viral spread by limiting the proliferation of infected cells. Gene ontology analysis has shown up-regulation of CREB3L1 gene during a ZIKV infection, suggesting CREB3L1 neuroprotective pathway is altered in nerve cells infected with ZIKV. The objective of our study is to assess the expression levels of CREB3L1 neuroprotective pathway during a ZIKV infection. SHSY-5Y neuroblastoma cells were differentiated with retinoic acid and infected with ZIKV at a multiplicity of infection (MOI) of 0.1 at different time points. After examining the cultures for cytopathic effects, a western blot assay was used to assess protein expression levels. Real-time quantitative reverse transcription polymerase chain reaction was performed to evaluate the gene expression of ZIKV viral load, CREB3L1 pathway targets genes CREB3L1, CAMKIV, INHBA, INHBE and SLC. Results indicate that viral load titers increased 12, 24 and 48 hours' post infection and that the infected cells began expressing cytopathic effects after 24 and 48 hours. However, the RNA and protein levels of CREB3L1 pathway did not show any differences in expression during a ZIKV infection, compared to controls, in a time dependent manner. Our results indicate that CREB3L1 genetic and protein expression levels are not altered during a 12, 24, 48 and 72 hour ZIKV infection. The expression of CREB3L1 pathway is not altered but may still be playing a role in infected cells as an antiviral response since cell death continues and viral load begin to decrease after 72 hours. The role of CREB3L1 in nerve cell survival processes and cell death assessment will be further evaluated in SHSY-5Y cells infected with ZIKV performing flow cytometry at different time points.

1092

THE EFFECT OF PRIOR ZIKA VIRUS INFECTION ON MARKERS OF MALE FERTILITY

Viviana Pinedo-Cancino[1], Filemon Bucardo[2], Jayrintzina Palacios[2], Hernan Vanegas-Solis[2], Maria Vasquez-Chasnamote[1], Rafael Saavedra-Langer[1], Saby Pinedo-Sotelo[1], Aravinda de Silva[3], Benjamin Leader[4], R. Matthew Coward[3], **Natalie M. Bowman**[3]

[1]*Universidad Nacional de las Amazonas Peruanas, Iquitos, Peru,* [2]*Universidad Nacional Autónoma de Nicaragua-León, Leon, Nicaragua,* [3]*University of North Carolina-Chapel Hill, Chapel Hill, NC, United States,* [4]*Reprosource, Inc., Marlborough, MA, United States*

Previously considered a minor febrile illness, novel characteristics of Zika virus (ZIKV) were identified during the 2015-2016 American epidemic - prolonged shedding in semen, sexual transmissibility, and congenital anomalies. In mouse models, ZIKV infection decreased testicular size and sperm production, resulting in significantly impaired fertility. ZIKV's effect in humans is not known, as only two small studies to date have reported a decline in sperm count and increased anomalies after acute ZIKV infection that recovered after several weeks. Fever itself can impair sperm production, confounding interpretation of these results. To investigate the long-term effect of ZIKV infection on human male fertility, we conducted a cohort study of young healthy men in two sites (Peru and Nicaragua) affected by the ZIKV epidemic. Approximately half of the population at each site was exposed to ZIKV, providing internal cases and controls. Men aged 18-40 years were enrolled at the start of the arbovirus transmission season. Demographic, clinical, and epidemiological data were collected at enrollment and updated quarterly. Men provided semen and blood quarterly for 6 months. Blood is tested for ZIKV EDIII IgG, dengue IgG/IgM, testosterone, and inhibin B. Fresh semen analysis was performed on-site; frozen semen is shipped to Reprosource, Inc. for Advanced Semen Report™ (ASR). Semen and serum markers of fertility are compared between ZIKV-seropositive and seronegative men. Data are analyzed by Student's t-test or Wilcoxon rank-sum test for continuous data and Chi-squared tests or generalized linear regression for categorical data. 110 men were enrolled (50 in Peru, 60 in Nicaragua). Mean age was 24 years. In Peru, 43% were seropositive for Zika. Preliminary data showed median sperm counts of 177-236 x 10^6, with 73% motility and 64-71% vitality. 4.1-6% of the sperm had normal morphology. Further serological and ASR tests are in process. This study will measure ZIKV's effect on male fertility and distinguish between temporary, fever-related declines in fertility and long-term virus-mediated damage.

1093

LAG-3: A POTENTIAL CHECKPOINT OF THE HUMORAL IMMUNE RESPONSE TO IMMUNIZATION

Brien K. Haun, Albert To, Teri Wong, Eileen Nakano, Lishomwa Ndhlovu, Axel T. Lehrer

The University of Hawaii, Honolulu, HI, United States

Emerging infectious viruses such as Zika virus (ZIKV) pose serious threats to human health. Currently, there are no FDA-approved therapeutics or vaccines available for ZIKV. Our laboratory has developed a vaccine

candidate using recombinant envelope protein (E) of the ZIKV. Our previous work has shown that neutralizing antibody responses are correlated with protection against viral challenge in BALB/c mice and non-human primates. Since lymphocyte-activation gene 3 (LAG-3) is an immune checkpoint receptor that directly interacts with MHC-II during antigen presentation, we decided to use a mouse model to investigate the effects of administering a LAG-3 blockade pre- and post-immunization to evaluate its effect on immunogenicity of the vaccine. We observed an increase in CD4+ T cells among the treatment group, unique T cell phenotypes, and differentially expressed genes. This alteration in immune cell populations was accompanied by an increase in vaccine potency based on ZIKV neutralizing titers. Based on preliminary observations, we believe that treatment with LAG-3 blockade with regard to immunization may modulate the magnitude of humoral responses by increasing T helper cell activity in germinal centers. Overall, we think that LAG-3 may be an important controller of the quality of adaptive immune responses and currently focus on investigating these phenomena in greater detail.

1094

IDENTIFICATION OF NOVEL YELLOW FEVER CLASS II EPITOPES IN YF17D VACCINEES

Daniela Weiskopf, Jose Mateo, Alba Grifoni, Hannah Voic, John Sydney, Alessandro Sette

La Jolla Institute for Immunology, La Jolla, CA, United States

Yellow fever virus (YFV) is a mosquito-borne member of the genus Flavivirus, which includes other important human-pathogenic viruses such as dengue, Japanese encephalitis, and Zika. Herein we report the identification of 129 YFV Class II epitopes in donors vaccinated with the live attenuated YFV vaccine. A total of 1156 peptides predicted to bind 17 different common HLA-DRB1 allelic variants were tested using IFNγ ELISPOT assays *in vitro* re-stimlulated PBMC from twenty-six vaccinees. Overall, we detected responses against 215 YFV epitopes. We found that the capsid and envelope proteins as well as the non-structural protein 3 (NS3) and NS5 were the most targeted proteins by CD4 T cells from YF-VAX vaccinated donor. In addition, we designed and validated by flow cytometry a CD4 mega pool composed of structural and non-structural peptides in an independent cohort of vaccinated donors. Overall this study provides a comprehensive prediction and validation of both structural and non-structural YFV epitopes in a cohort of YF17D vaccinated individuals. With the design of a CD4 epitope MP we further provide a useful tool to detect ex vivo responses of YFV-specific CD4 T cells in small sample volumes.

1095

CANONICAL PRR SIGNALING PATHWAYS ARE NOT VITAL IN THE ANTIVIRAL RESPONSE AGAINST ZIKV INFECTION IN HUMAN SERTOLI CELLS

Boonyanudh Jiyarom[1], Daniel P. Strange[1], Nataliya Panova[1], Pei-Yong Shi[2], Michael Gale Jr.[3], Saguna Verma[1]

[1]*University of Hawaii at Manoa, Honolulu, HI, United States,* [2]*University of Texas Medical Branch, Galveston, TX, United States,* [3]*University of Washington School of Medicine, Seattle, WA, United States*

Zika virus (ZIKV) is shown to establish persistence in the testes; however, the mechanisms remain undefined. We recently demonstrated that ZIKV was able to replicate and persist in human Sertoli cells (SC), an important cell type of seminiferous tubules. In addition, our host transcriptomic data predicted RIG-I and TLR3 as the top virus sensing pathways modulated in SC. Here, we elucidate the association of ZIKV replication with the activation of critical antiviral pathways. RIG-I, TLR3, MAVS, and TBK1 were inhibited using siRNA or small molecule inhibitors in SC and infected with ZIKV PRVABC59. A549 and human brain endothelial cells (HBMEC) were used as controls. Data demonstrated that while inhibition of these signaling molecules in A549 and HBMEC increased virus by >50%, we did not observe similar response in SC. However, gene expression of IFNB, MxA, and IFIT1 was reduced in the PRR deficient SC. Further, we observed that exogenous IFNβ treatment significantly reduced ZIKV titers in SC that was comparable to A549, thus indicating that IFN signaling is active in SC. To delineate the crosstalk of PRRs with other immunosuppressive pathways in the testes, SC were pretreated with TGFβ and virus titers measured. Interestingly, we observed an increase in ZIKV titers in TGFβ treated SC. Our data suggest that despite being activated, canonical virus sensing pathways do not control ZIKV replication in SC. A possible explanation might be that activation of other regulators of innate immune pathways that are active in SC such as TGFβ may interact with these PRRs and facilitate virus persistence. Collectively, these results fill in gaps in our understanding of the antiviral signaling mechanisms underlying ZIKV infection and persistence in human testes.

1096

INSECT-SPECIFIC FLAVIVIRUS HOST RANGE AND TRANSMISSION

Stephen A. Peinado, Lyric Bartholomay

University of Wisconsin – Madison, Madison, WI, United States

The Genus *Flavivirus* is a diverse group of viruses that include species that have had a significant impact on human health worldwide both historically and in modern times. The insect-specific flaviviruses (ISFs) phylogenetically align into the Genus, but only infect insect hosts and do not infect vertebrate cells. While numerous ISFs have been described, little information is known about the limitations to their host range in insects, and much remains to be explored regarding potential modes of transmission. Using Aedes flavivirus (AEFV) as a model ISF, we investigated the host range across multiple genera of mosquitoes as well as vertical, venereal, and *per os* routes of transmission. Mosquitoes were intrathoracically injected with 1000 genome copy equivalents of AEFV and tested for infection by RT-PCR at 7 dpi. *Aedes (Ae.aegypti, Ae. triseriatus, Ae. albopictus)* and *Toxorhynchites amboinensis* tested were highly permissive to infection, while *Culex pipiens* and *Anopheles gambiae* displayed low prevalence of infection. To determine the rate of vertical transmission for AEFV, eggs from a persistently infected colony of *Ae. albopictus* were hatched and reared to adults. Male and female adult F_1 from each egg sheet were tested for infection by RT-PCR and revealed complete (100%) transovarial and filial transmission in both male and female F_1 individuals. To evaluate *per os* infection potential, we exposed adult and larval mosquitoes to AEFV. Adult female *Ae. aegypti* were provided a 1:1 solution of 10% sucrose and virus stock then tested for infection 7 dpe for infection by RT-PCR. Similarly, adult female *Ae. aegypti* were provided a 1:1 solution of defibrinated sheep blood and virus stock. To evaluate ingestion of infected cells as a possible infection route, uninfected 1st instar *Ae. aegypti* larvae were allowed to feed on C6/36 cells infected with AEFV. Larvae were allowed to develop into adults and tested individually for infection. The results of these trials expand our understanding of ISFs, suggest some plasticity in host range among mosquitoes, and underscore the importance of vertical transmission for the ISFs to persist in nature.

1097

SEROCONVERSION RATES OF TORCH PATHOGENS IN WOMEN PARTICIPATING IN A ZIKA PREGNANCY COHORT STUDY IN MOMBASA, KENYA FROM 2017-2019

Elizabeth Anne Hunsperger[1], Eric Osoro[2], Peninah Munyua[1], M. Karuiki Njenga[2], Harriet Mirieri[2], Gilbert Kakwai[3], Dennis Odhiambo[3], Moshe Alando[3], Victor Omballa[3], Marc-Alain Widdowson[4], Irene Inwani[5]

[1]*Centers for Disease Control and Prevention, Nairobi, Kenya,* [2]*Washington State University, Nairobi, Kenya,* [3]*Kenya Medical Research Institute, Nairobi, Kenya,* [4]*Institute of Tropical Medicine, Antwerp, Belgium,* [5]*Kenyatta National Hospital and University of Nairobi, Nairobi, Kenya*

Women infected during pregnancy with TORCH pathogens (Toxoplasmosis, Other, Rubella, Cytomegalovirus, and Herpes simplex viruses) have a higher risk of adverse birth outcomes because of the potential for mother to baby

transmission. Adverse birth outcomes include, among others, stillbirth and miscarriage. To address this risk, we collected serum specimens from a pregnancy cohort study aimed to detect Zika virus (ZIKV) in Kenya in three health facilities in Mombasa county. A random sample of 255 participants were selected for TORCH pathogen testing on enrollment and delivery samples. These sera were tested by enzyme linked immunosorbent assay (ELISA, Euroimmun) for 5 TORCH pathogens. IgG seroconversion was defined as a negative test result at enrollment and a positive test result at delivery. Also, previous TORCH pathogen exposure was determined by IgG seropositivity at enrollment. A total of 2312 pregnant women were enrolled in a ZIKV pregnancy cohort from 2017 to 2019 with monthly serum collections. The median age was 28 years (range 18-42) and the median gestation at enrollment was 20 weeks (range 5-28). Eighty eight percent of the participant had live births, 7.8% had still birth, 3.5% had an abortion (<22 weeks gestation). The ELISA results of the 5 TORCH pathogens tested to date (Toxoplasma gondii, treponema pallidum, HSV-1, Bordetella pertussis and chlamydial trachomatis) had a seroconversion rate of 2.5% , 3.1% , 1.6% , 0% and 2% and the prevalence of previous infection (IgG positive at enrollment) was 16.2% , 10.2% , for 96.1% , 3.8% and 38.8% respectively. TORCH testing determined the prevalence and seroconversion for five different pathogens that can be compared to other low- and middle-income countries in Africa. Further analysis is needed to better understand the correlation of seroconversion of TORCH pathogens to adverse birth outcomes from this cohort.

1098

THE MEASLES-VECTORED LASSA VACCINE MV-LASV IS SAFE AND IMMUNOGENIC - INTERIM RESULTS FROM A FIRST IN MAN PHASE 1 CLINICAL TRIAL

Sylvain Baize[1], Yvonne Tomberger[2], Roland Tschismarov[2], Erich Tauber[2], **Katrin Ramsauer**[2]

[1]*Institut Pasteur, Lyon, France,* [2]*Themis Bioscience, Vienna, Austria*

Lassa fever is a viral hemorrhagic fever caused by Lassa virus, which is endemic in large parts of West Africa and causes annual outbreaks with seasonal peaks during the dry season. The spectrum of disease ranges from asymptomatic and mild to severe and life-threatening clinical disease, with an estimated mortality of up to 30% in hospitalized patients. With high numbers of infected individuals and limited measures for disease control, LF poses a major health and economic burden on affected regions. Consequently, WHO has included LF on its Blue Print list of pathogens requiring urgent efforts in research and development and the Coalition for Epidemic Preparedness Initiative (CEPI) has recently provided funding for the clinical development of promising vaccine candidates. The measles-vectored vaccine MV-LASV was previously shown to be well-tolerated, immunogenic and efficacious in NHP, and was thus moved to clinical phase 1 evaluation in healthy volunteers. Here, we report interim data from this trial, showing that MV-LASV has an acceptable safety and tolerability profile and induces both humoral and cellular immunity in trial participants.

1099

SURVEILLANCE OF NEURAMINIDASE INHIBITION SUSCEPTIBILITY OF INFLUENZA A VIRUS (IAV) ISOLATES OBTAINED FROM KENYA, 2008 TO 2017 INDICATED MIXED SENSITIVITIES

Meshack Wadegu, Silvanos Opanda, Janet Majanja, Rachel Achilla, James Njiri, Wallace Bulimo

USAMRU-KENYA, Nairobi, Kenya

Neuraminidase inhibitors have become the main antiviral agents useful in mitigation of IAV infections. Data on NAI susceptibility profile of influenza A isolates circulating within the Eastern African region remains scanty. Here we characterized the NAI susceptibility of the 2008-2017 influenza A strains circulating in Kenya, by profiling known molecular markers in neuraminidase (NA) protein. Global NA and HA sequences were selected from Gene bank for inclusion in the alignments together with local IAV sequences prior to phylogenetic reconstruction using the Bayesian method of tree inference. During the study period 2008 to 2017 molecular analyses involving- 75HA and 84NA IAV H1N1pdm09; 100HA and79NA IAV H3N2; 26HA and 33NA seasonal IAV H1N1-genetic fragments was carried out to investigate their susceptibility to oseltamivir. Majority of the seasonal influenza A/H1N1 strains obtained in the 2008-2009 season possessed H275Y marker of Oseltamivir drug resistance. The 2008 IAV H1N1 strains obtained in Kenya belonged to clades 2B.I while the 2009 strains were of the Northern European lineage (clades 2B.II) the dual signature H275Y and D354G substitutions. All the A/H1N1pdm09 strains obtained from Kenya from 2009-2017 were cluster 2 viruses possessing the main substitution S203T. IAV H1N1pdm09 strains obtained from Kenya, 2009-2017 lacked H275Y substitution. The IAV H3N2, HA phylogeny indicated that the 2012-2013 strains were Brisbane/10/2007-like possessing S144N substitution. Most of the 2016 IAV H3N2 Kenyan strains belonged to Perth/16/2009 clades defined by S/N144K substitution. The 2017 virus strains possessed 144S mutation same as the current vaccine strain Hongkong_4801_2014. All the Kenyan IAV H3N2 strains analyzed lacked R292K, Q136KD151V/D and N294S substitutions. Most of the seasonal influenza A/H1N1 strains, 2008-2009 season obtained from Kenya possessed H275Y marker. The IAV H1N1pdm09 and IAV H3N2 strains obtained from Kenya have remained susceptible to Oseltamivir. Targeted NAI surveillance is important in timely detection and control of variant strains of great pandemic potential.

1100

ANTIBODY-MEDIATED IMMUNITY IN EBOLA VIRUS DISEASE SURVIVORS AND THEIR HOUSEHOLD CONTACTS

Kayla R. Shore[1], Iris Bica[1], Augustine Goba[2], Lansana Kanneh[2], Mambu Momoh[2], Robert Samuels[2], Ashley Smira[1], Nell Bond[1], Donald Grant[2], James Robinson[1], John S. Schieffelin[1]

[1]*Tulane University, New Orleans, LA, United States,* [2]*Kenema Government Hospital, Kenema, Sierra Leone*

Zaire ebolavirus (EBOV), the causative agent of Ebola Virus Disease (EVD), is highly lethal, with case fatality rates reaching 90%. Previous EBOV seroprevalence studies have found rates between 1.4% and 15.3% throughout regions of western and central Africa. Studies investigating seropositivity in household contacts (HCs) of known EVD survivors have produced variable results, with rates ranging from 1.4% to 48%. The estimated secondary attack rate for those with direct contact is 22.9%, indicating many studies may be underestimating seroprevalence. Furthermore, few studies have investigated the efficacy of antibodies present, and none have looked at this measure in HCs. Taken together, there is an indicated need for increased understanding of the humoral immune response following EVD to better inform therapeutic and vaccine development. This study aims to determine the rate of IgG seropositivity in EVD survivors and their HCs and investigates the neutralization capacity of IgG seropositive individuals. Survivors were considered eligible if they had been discharged from an Ebola Treatment Unit as recorded by the Sierra Leone Association of Ebola Survivors. Serum samples were collected from EVD survivors and up to 3 of their HCs, with sera from 212 survivors and 490 HCs available for testing. Sample seropositivity was determined using both GP and VP40 ELISAs. 77.8% of survivors and 31.6% of HCs were anti-GP IgG positive based on ELISA results ($p<0.001$). Positive samples and a subset of negative samples were then tested for neutralizing antibodies using a pseudovirus expressing Ebolavirus GP and luciferase. 79.05% of survivors developed a response capable of neutralizing >50% of the pseudovirus, whereas only 7.64% of household contacts were considered neutralizing ($p<.001$). The results of this study suggest that most survivors mount anti-GP responses over the course of EVD, in agreement with findings in the growing body of literature on EVD. However, the small number of survivors capable of producing a strong neutralization response (24.29%) indicate that antibody-mediated protection may not be necessary for survival of disease.

1101

ABSENCE OF SARS-COV-2 IN RESPIRATORY SAMPLES OF PATIENTS WHO PRESENTED WITH INFLUENZA-LIKE ILLNESS IN WESTERN CAMBODIA

Mariusz Wojnarski[1], Darapiseth Sea[1], Horm Srey Viseth[2], Phalla Y[2], Veasna Duong[2], Philippe Dussart[2], Nichapat Uthaimongkol[1], Samon Nou[3], Tippa Samon.Nou.ctr@afrims.org[1], Sovannarith Em[3], Brian Vesely[1], Piyaporn Saingam[1], Paphavee Lertsethtakarn[1], Norman Waters[1], Stefan Fernandez[1], Philomena Raftery[4], Michael Kinzer[5], Seng Heng[6], Ly Sovann[6], Chanthap Lon[1], Erik A. Karlsson[2]

[1]*Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand,* [2]*Institut Pasteur du Cambodge, Phnom Penh, Cambodia,* [3]*Armed Forces Research Institute of Medical Sciences, Phnom Penh, Cambodia,* [4]*World Health Organization, Phnom Penh, Cambodia,* [5]*US Centers for Disease Control and Prevention, Phnom Penh, Cambodia,* [6]*Communicable Disease Control Department, Phnom Penh, Cambodia*

In addition to active case finding, leveraging existing national and sub-national influenza surveillance systems can result in efficient and cost-effective implementation of COVID-19 surveillance in Cambodia. It is critical to enhance surveillance of transmission in the community, particularly among patients with influenza-like illness (ILI) symptoms, since influenza and COVID-19 have similar clinical presentations. Due to limited access to diagnostic services in rural areas of Cambodia, it is unclear if COVID-19 has circulated undetected in these underserved areas. Therefore, the incidence of SARS-CoV-2 in patients who presented with influenza-like symptoms was assessed at select ILI-surveillance sites to help guide local policies on testing in the midst of pandemic and to assess the risk to health care workers collecting nasopharyngeal swabs under approved ILI research protocols. Out of 200 samples collected between Jan 1, 2020 to March 31, 2020, 28% were positive for influenza type A and 0% tested positive by RT-PCR for SARS-CoV-2. Most patients presenting with ILI symptoms (91%) were less than 18 years if age. We also assessed the prevalence of human coronaviruses HKU1, NL63, OC43, and 229E in 1,735 samples collected from 2014-2020 in Cambodia by multiplex RT-PCR, with only 73 samples (4%) being positive. Despite concerns about cross-reactivity, based on the low prevalence of non-SARS coronaviruses in our study population, they are unlikely to be a major cause of COVID-19 false positive results on rapid tests when deployed. There is no evidence from our dataset that COVID-19 cases are being undiagnosed in patients presenting with influenza-like illness in western Cambodia. The risk to health care workers who collected ILI samples was low in our study population given lack of positive samples for SARS-CoV-2. The ILI sites which are already established can be utilized for ongoing surveillance of COVID-19 in rural, western Cambodia. Screening of additional nasopharyngeal samples for SARS-CoV-2 is ongoing and these samples will be processed at the reference laboratory, Institute Pasteur in Cambodia.

1102

REPORTED CASES OF RABIES IN WILDLIFE AND LIVESTOCK IN MONGOLIA FROM 2012-2018

Graham Matulis[1], Altantogtokh Doniddoniddemberel[2], Paul Lantos[3], Jordan Jones[1], Tsogbadrakh Nymadorj[2], Bayar Tserendovdon[2], Ganzorig Sainkhuu[2], Michael E. von Fricken[1]

[1]*George Mason University, Fairfax, VA, United States,* [2]*National Center for Zoonotic Diseases, Ulaanbaatar, Mongolia,* [3]*Duke University School of Medicine, Durham, NC, United States*

Rabies is a neglected tropical disease with poorly characterized epidemiology within Mongolia. An estimated 2,000 people receive post-exposure prophylaxis for rabies every year in Mongolia, with exposures most often reported from dogs, foxes, and wolves. Less than 20% of domestic dogs are reported to be vaccinated, while little to no immunization of wild carnivores occurring in Mongolia. This study sought to analyze rabies cases occurring in both domestic and wild animals in hopes to better characterize the disease ecology of rabies throughout Mongolia. Using data collected between 2012-2018 from the National Centre for Zoonotic Diseases, cases were analyzed with respect to the animal type and distribution throughout Mongolia's aimags (provinces). Cattle represented the most frequently reported domestic animal and was the animal with the highest number of cases in 14 aimags. The fox was the most reported wild animal with rabies, representing the highest wild animal count in 10 aimags. The number of rabies cases was distributed among all 21 aimags, with the Khuvsgul, Uvurkhangai, and Govi-Altai aimag reporting the highest animal rabies counts. Dogs represented 9.34% of total animal cases and was the highest reported carnivore in 5 aimags. Interestingly, the Govi-Altai aimag reported the highest proportions of both dog and wolf cases, possibly representing a unique ecological interface between predators within this aimag. This study demonstrates how widely dispersed rabies cases are in both domestic animals and wildlife throughout Mongolia. While foxes were determined to be the most reported carnivore with rabies in Mongolia, the counts in certain aimags indicated the possible importance of wolves and dogs in rabies transmission in specific locations. This study provides valuable information about the geographic distribution and disease ecology of rabies within Mongolia.

1104

HOW U.S. PUBLIC UNIVERSITIES RESPONDED TO THE COVID-19 PANDEMIC IN MARCH 2020: LESSONS LEARNED FROM THE VARIATIONS IN TIMING OF KEY DECISIONS

Kevin E. Cevasco, Hayley North, Sheryne Zeitoun, Abigail F. Gregory, Rachel N. Wofford, Maha H. Hassan, Graham A. Matulis, Aya D. Abdo, David Farris, Amira A. Roess, Michael E. von Fricken

George Mason University, Fairfax, VA, United States

To examine how and when public universities responded to the U.S. COVID-19 outbreak, regarding decisions to cancel international travel, switch to online learning, transition faculty and staff to remote work, limiting on-campus housing, and implementing campus closures. Announcements for 412 U.S. public universities were compiled targeting COVID-19 university decisions between February 27 and March 31, 2020Substantial heterogeneity in decision making and the timing of those decisions as they relate to key state, national, and global emergency announcements were observed. The WHO pandemic declaration coincided with announcements to move away from on-campus learning. Universities decisions were made largely at the university level and not coordinated by government agencies, leading to staggered announcements and major variations in university timelines between states and within states. The importance of synchronizing university decisions in a national emergency scenario cannot be understated. Dissonant university decisions and announcement dates may potentially lead to mixed messaging and a reduction of the effectiveness of early interventions. Clear guidance is needed moving forward regarding university operations for fall and summer.

1105

MACHINE LEARNING AND LIGAND-BASED APPROACHES FOR DRUG REPURPOSING AGAINST COVID-19

Emna Harigua-Souiai[1], Yosser Zina Abdelkrim[1], Arnaud Blondel[2], Ikram Guizani[1]

[1]*Institut Pasteur de Tunis, Tunis, Tunisia,* [2]*Institut Pasteur, Paris, France*

The COVID-19 pandemic caused by SARS-CoV-2 raises global health concerns, as there are currently no vaccine or valid treatment against this infectious disease. Given the urgency and to overcome time-consuming drug discovery process, drug repurposing is the main focus of multiple research groups to identify treatments. We herein propose a hybrid approach that makes use of information on published compounds known to have antiviral effects on coronaviruses and the FDA approved drugs collection to propose potential effectors against SARS-CoV-2, in particular. For this purpose, we conducted an extensive literature search to retrieve data on molecules with validated effects on SARS-CoV, SARS-CoV2 and/or MERS viruses. Data on the chemical structure encoded into SMILES and

available data on enzymatic or antiviral effects were also collected. Using the RDkit library, we implemented a pipeline that calculates a multitude of molecular descriptors of the anti-coronavirus set of molecules on one hand, and the FDA approved drugs collection on the other hand. These descriptors were used to appreciate the chemical diversity of the antiviral dataset, then to select the FDA approved drugs presenting significant chemical similarity with the anti-viral molecules using a threshold. Then, we used these molecular descriptors to train machine learning algorithms (ML) such as Support Vector Machine and Random Forests to predict a set of approved drugs with anti-viral potential. We identified 264 antiviral molecules described in the literature since the SARS-CoV outbreak in 2003. Based on the chemical descriptors, we selected 74 FDA approved drugs that are chemically similar to the antiviral molecules. The ML algorithms were implemented, and their validation is ongoing, to build an 'antiviral profile' to predict which of the FDA approved drugs will likely be active against the SARS-CoV-2. The retained molecules will be tested for their anti-viral growth effects. The dataset will be shortly made available as a contribution to the global scientific efforts of drug discovery against COVID-19.

1106

PREPARING FOR THE COVID-19 PANDEMIC RESPONSE IN A COUNTRY EMERGING FROM AN EBOLA EPIDEMIC: ASSESSMENT OF HEALTH WORKERS' KNOWLEDGE AND ATTITUDES ON COVID-19 IN GUINEA

Abdoulaye Touré[1], Ibrahima Camara[1], Alioune Camara[2], Salifou Talassone Bangoura[1], Frederic Le Marcis[3], Alpha Kabinet Keita[1]

[1]*Center of Research and Training of Infectious Diseases, Gamal Abdel Nasser University of Conakry, Conakry, Guinea,* [2]*Department of Public Health, Gamal University of Conakry, Conakry, Guinea,* [3]*Department of Social Sciences, Research Team LADEC, Ecole Normale Supérieure de Lyon, Lyon, France*

On January 30, 2020, the World Health Organization designated the COVID-19 outbreak as a Public Health Emergency of International Scope. The purpose of this study was to assess the knowledge and attitude of health care workers about the prevention of COVID-2019 in Conakry, Guinea. This was a cross-sectional analytical study carried out from February 1st to 29th, 2020 among 548 frontline health care workers in 11 health facilities (3 health centres, 5 communal health centres, 3 national hospitals) in Conakry. Data on socio-demographic characteristics, knowledge, attitudes of prevention and control of COVID-19 were collected by administering a standardized questionnaire. Knowledge and attitude scores were calculated and dichotomized: poor (score < 50%) and good (score ≥ 50%). Logistic regression models were conducted to identify factors associated with good knowledge or good attitude. The median age of health workers surveyed was 29 years (interquartile range: 25-38). Most health workers surveyed were female (57.1%); 28.1% were nurses and 23.7% were recruited at Donka National Hospital. The proportion of health care workers with good knowledge and attitudes was respectively 70.6% and 57.7%. Even though the majority (57.5%) of health personnel had already received training in infection prevention and control, infection prevention and control (ICP) practices were not systematically applied, as the rate of application was less than 80% for each module. Factors associated with good knowledge of COVID-19 were female sex [Adjusted Odds Ratio (AOR): 2.5: 95% Confidence Interval (CI) 1.6 to 3.7], working in the Matam Communal Health Centre (OR= 1.99, 95% CI [1.13 -3.46] and pharmacist occupation (OR=9.83 95% CI [1.09-38.44]). No reported history of Ebola cases in the ward (OR=1.57, 95% CI [1.09-2.25] was the only factor associated with poor attitude. This study highlights the need of preparation of health staff immediately after the announcement of an outbreak. This specific preparation will facilitate the implementation of prevention and protection measures against COVID-19.

1107

ESTIMATION AND PREDICTION OF THE NEED FOR POST-EXPOSURE PROPHYLAXIS IN CAMBODIA USING SPATIAL BAYESIAN REGRESSION MODELLING

Jerome Nicholas Baron[1], Veronique Chevalier[2], Sowath Ly[3], Philippe Dussart[3], Didier Fontenille[3], Malen Chan[3], Beatriz Martinez-Lopez[1]

[1]*University of California Davis, Davis, CA, United States,* [2]*CIRAD, Universite de Montpelier, Montpelier, France,* [3]*Institut Pasteur du Cambodge, Phnom Penh, Cambodia*

In rabies-endemic Cambodia, each year, some 20,000 patients come to the Institut Pasteur du Cambodge (IPC) following a dog bite or other animal related injury, to seek post-exposure prophylaxis (PEP). Most patients come from Phnom Penh and its surroundings, where IPC is located, likely underestimating the true need for PEP in more rural areas distant from the capital. This study aims to assess the need for PEP by taking into account the spatial heterogeneity of the data and accessibility to the PEP center in Phnom Penh. We used spatial Bayesian Poisson regression to model prospective PEP patients in each spatial unit under study. The data include IPC records of some 290,000 individuals that came to IPC from 2000 to 2016 with location, as well as demographic and socio-economic census data. Accounting for travel time to IPC and using a conditional autoregressive structure, we adjusted for the unequal distribution of observations. The PEP infrastructure in Cambodia is currently being expanded, so we modelled different scenarios taking into account accessibility to new centers currently being opened or under development. We compared models at different spatial scales (provinces and districts) to choose the best fitting model and help predict ideal locations for future centers. Outcomes were translated into geographical estimates and risk maps. These results were used together with another study looking at the probability of a PEP patient being exposed to a rabid animal in each province to estimate the burden of untreated human rabies in Cambodia. Preliminary results confirm that distance to IPC is strongly associated with the likeliness of an individual seeking PEP, leading to likely underestimations of the need in provinces distant from Phnom Penh. Distance- and population density-adjusted predictions confirmed empirical knowledge that certain provinces seem to have lower reporting than would be expected on average if PEP access was not centralized. This study is part of a broader effort to model rabies and interventions in Cambodia, helping to determine resource allocation, risk-based strategies, and guide policies to meet eradication targets.

1108

SARS-COV-2 TRANSMISSION PARAMETERS AMONG COVID-19 CASES IN NORTH CAROLINA

Raquel A. Binder[1], Natalie Alarja[1], Emily Robie[1], Kara Kochek[1], Leshan Xiu[1], Lucas Rocha Melogno[1], Sumana Goli[1], Anfal Abdelgadir[1], Amanda Farrell[1], Abby Turner[1], Cassandra Lautredou[1], Benjamin D. Anderson[2], Gregory C. Gray[1]

[1]*Duke University, Durham, NC, United States,* [2]*Duke Kunshan University, Kunshan, China*

A pneumonia-like illness outbreak was traced to a novel coronavirus (SARS-CoV-2) in December 2019. Containment has not been successful and COVID-19 (SARS-CoV-2 disease) spread rapidly. To date there have been over 2 million cases and over 128,000 deaths globally. In North Carolina, SARS-CoV-2 was first detected on March 3rd 2020 and currently counts 7,529 cases and 266 deaths. Better understanding of the viral and epidemiological hallmarks are needed to develop interventions. The present study seeks to gather SARS-CoV-2 infection parameters as well as to identify risk factors for infection by enrolling up to 250 confirmed and hospitalized COVID-19 cases at the Duke University Hospital, along with their self-referred close contacts (maximum of 1,500 total participants). Biological samples (nasopharyngeal [NP] swab, rectal swab, saliva, and serum) and questionnaires will be collected during the acute and convalescent phase (day 14 and day 28 post-enrollment) for hospitalized

cases. For close contacts the same samples will be collected during enrollment and follow up visit (day 21 post-enrollment). Furthermore, bioaerosol samplers are set up and fomites collected in the room of hospitalized cases. The study was initiated on April 10th 2020 and currently encompasses 6 cases and 4 close contacts. Among the analyzed acute samples of 5 cases, 2 had positive NP and rectal swabs and 2 had positive NP swabs and saliva specimens, based on the CDC RUO SARS-CoV-2 molecular assay. Over time the study aims to (i) determine the prevalence of potentially aerosolized SARS-CoV-2 by keep collecting aerosol samples in the hospital room of confirmed COVID-19 patients; (ii) characterize symptoms, incubation period, and secondary attack rate of COVID-19 cases and their close contacts; (iii) determine risk factors for SARS-CoV-2 infection; (iv) determine prevalence of SARS-CoV-2 co-infection and other circulating respiratory viruses during the study period; (v) determine the prevalence of SARS-CoV-2-positive fomites in a hospital room setting; and (vi) characterize and sequence circulating SARS-CoV-2 strains.

1109

CHARACTERIZING DIFFERENCES ACROSS LIBRARY PREPARATION METHODS FOR WHOLE GENOME SEQUENCING OF SARS-COV-2 VIA ILLUMINA PLATFORM

Khajohn Joonlasak[1], Insee Sensorn[2], Arporn Wangwiwatsin[3], Krittikorn Kumpornsin[4], Elizabeth Batty[5], Wudtichai Manasatienkij[1], Angkana Huang[1], Stefan Fernandez[1], Anthony R. Jones[1], Chonticha Klungthong[1], Theerarat Kochakarn[6], Namfon Kotanan[6], Bhakbhoom Panthan[2], Ekawat Pasomsub[7], Treewat Watthanachockchai[7], Kingkan Rakmanee[7], Thanat Chookajorn[6], Wasun Chantratita[2]

[1]*Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand,* [2]*Center for Medical Genomics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand,* [3]*Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand,* [4]*Wellcome Sanger Institute, Hinxton, Cambridgeshire, United Kingdom,* [5]*Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand,* [6]*Genomics and Evolutionary Medicine Unit (GEM), Centre of Excellence in Malaria Research, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand,* [7]*Virology Unit, Department of Pathology, Faculty of Medicine, Ramathibidi Hospital, Bangkok, Thailand*

The 2019 novel coronavirus disease (COVID-19) pandemic has become a global health crisis. In order to perform real-time genomic surveillance of SARS-CoV-2, the etiological virus of COVID-19, multiplexed PCR primer sets for whole-genome sequencing (WGS) were developed by the ARTIC network to prepare amplicon sequencing libraries for Illumina-based platforms. Subsequently, a comparison of sequences derived from three library preparation methods (Nextera XT, Nextera Flex and KAPA HyperPreps kit) was done on six SARS-CoV-2 RT-qPCR positive samples (Ct values: 18-26) from multiple lineages, likely to have originated from China and European countries, and from Thai-specific lineages branched out from Clade A.2, which is suspected to be linked to the transmission chains from local boxing stadiums. The sequence coverage from every method exceeded 99.45% with median depth of coverage (DOC) values of >8,000 from the KAPA HyperPreps kit, followed by >5,400 from the Nextera Flex kit, and finally >3,700 from the Nextera XT kit. Additionally, percentage of positions that lack ≥10 supporting reads with a Q-score ≥20 were ≤1.93, 1.07, and 0.74, respectively. Amplicon dropouts were observed in all methods, but KAPA HyperPreps kit data was superior due to better coverage at amplicon dropouts. However, the 5 hrs spent to execute the KAPA workflow took longer than the Nextera kits which clocked in at 3 hrs for the Flex and 2.95 hrs for the XT. Ultimately, features such as depth of coverage, coverage at amplicon dropouts, and processing time should all be considered when selecting a sequencing pipeline for SARS-CoV-2.

1110

CASE REPORT: A DIABETIC, HYPERTENSIVE, OBESITY AND COVID19 INFECTION PATIENT AT IQUITOS, PERU

Jean Nikolae Hernández[1], Carlos Alvarez[2], Falgunee K. Parekh[3]

[1]*University of Puerto Rico, San Juan, Puerto Rico,* [2]*Regional Direction of Loreto, Iquitos, Peru,* [3]*EpiPointe in Cary, North Carolina, NC, United States*

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel virus identified in Wuhan, China in November 2019 causing COVID19 pandemic. In Peru, the first COVID 19 case was reported in March 6th, 2020. Patients who are elderly and have underlying conditions are reported as having more severe illness and increased risk of mortality from COVID-19. Here we report on a confirmed case of COVID-19 from Iquitos, Peru, which is a city of Peruvian Amazon that is endemic for malaria, dengue, leptospirosis and many other tropical diseases. Currently in Iquitos there are 585 confirmed cases of COVID-19 and 26 deaths. A 62 years old man, with history of diabetes mellitus diagnosed in 2005 and receiving regular treatment with metformin and glibenclamide. The patient has a BMI of 32.43, and has had hypertension since 2009, but was not under treatment. The patient experienced low fever, malaise, sore and itchy throat, and lumbar pain, and was confirmed positive for SARS-Cov2 by RT-PCR on March 27, four days after symptoms began. The patient did not receive medical treatment, only hydration and isolation at home. The protocol for follow up was by cell phone calls for 14 days, asking about signs and symptoms, until symptoms were cleared. The patient recovered and was positive for IgG antibodies for SARS-Cov2 by serological test on April 10. Despite his older age and underlying conditions, the patient experienced mild COVID-19 disease. Additional data on mild COVID-19 cases in vulnerable populations is needed to understand the mechanisms of recovery in the presence of underlying conditions.

1111

UTILIZING EBOLA VIRUS GLYCOPROTEIN SUBUNITS TO REVEAL ANTIBODY POPULATIONS ELICITED BY A RECOMBINANT SUBUNIT EBOLA VIRUS VACCINE

Aquena Ball, Axel Lehrer

University of Hawaii at Manoa, Honolulu, HI, United States

Ebola virus (EBOV) causes lethal hemorrhagic fevers with case fatality rates of up to 90%. The recent large outbreak in West Africa and current outbreak in the DRC demonstrate an obvious need for vaccines and therapeutics. All vaccine candidates use the surface glycoprotein as the main antigen. However, the method of protection induced by the EBOV glycoprotein remains unclear. The EBOV glycoprotein is composed of GP1 and GP2 linked by a disulfide bond. GP1 contains the receptor binding domain, glycan cap, and the heavily glycosylated mucin-like domain while GP2 contains the fusion loop and transmembrane anchor. We have developed a subunit vaccine that allows for an increased safety profile as well as shows increased thermostability, which allows easier deployment in central Africa. With our studies we expect to reveal antibody patterns differentiating survivors and non-survivors from non-human primate studies as well as reveal subdomains that are more protective and/or immunodominant to guide future vaccine development as well as provide insights into correlates of protection. Subunits of the EBOV glycoprotein have been produced in Drosophila S2 cells including full length GP, GPΔmucin-like domain, GP1, GP2, and sGP. These subdomains will be used to explore the binding patterns of antibodies elicited by a highly purified GP subunit vaccine. Ongoing analysis focuses on samples from non-human primates immunized with different doses of EBOV GP + adjuvant that will be analyzed for antigen binding IgG to differentiate the pre-challenge antibody binding patterns to the different EBOV subdomains.

1112

MODELING THE IMPACT OF MASS MIGRATION ON VIRAL HEPATITIS PREVALENCE AND FUTURE DISEASE BURDENS

Kenneth A. Valles, Lewis R. Roberts

Mayo Clinic, Rochester, MN, United States

Globally, over 300 million people have chronic viral hepatitis (CVH) and the resulting annual mortality exceeds that of malaria, tuberculosis, or HIV. CVH is the primary driver of hepatocellular carcinoma (HCC), and despite international efforts, CVH and HCC prevalences continue to climb as the long-term sequelae abate global health. Migrants remain a vulnerable group impacted by CVH and an understanding of the disease burdens and outcomes is key to achieving hepatitis elimination and stopping the growing epidemic. This study aims to estimate recent changes in HBV and HCV prevalence and the projected outcomes of cirrhosis and hepatocellular carcinoma among migrants to select high-immigrant nations. Migration values and demographics from 2013-2018 were obtained from international databases for destination countries. Country-of-birth HBV and HCV prevalence were obtained for the origin nations from the Polaris Observatory and systematic reviews. Disease progression estimates were generated using established rates (AASLD, EASL) and peer-reviewed literature when needed. Of the 552,143 documented migrants arriving between 2013 and 2018, 21,228 were estimated to have chronic HBV and 11, 695 to have chronic HCV. Prevalence of HBV and HCV by migrant cohort, and resulting host-nation prevalence, were determined (highest, Sweden +84% HBV). Forecasted cases of cirrhosis, decompensation, and HCC were estimated to be 6,252, 1,745, and 1,259 respectively. Large-scale migration is a considerable factor in the global CVH and HCC landscape. The prevalence and preventable long-term sequelae are expected to increase in high-immigration nations and these increases are likely to be highest among migrant populations. Given the latency of progression to end-stage liver disease and cancer there is an existing, albeit narrowing, window to identify the recent migrants at greatest risk and intervene, reducing morbidity, mortality, and future treatment costs. The results from this study offer key country-of-origin and age cohort insight into high-risk groups deserving of prioritized screening and treatment.

1113

SEROLOGY AND BEHAVIORAL PERSPECTIVES ON EBOLA VIRUS DISEASE AMONG BUSHMEAT VENDORS IN EQUATEUR, DEMOCRATIC REPUBLIC OF THE CONGO, AFTER THE 2018 OUTBREAK

Ashley Lucas[1], Charles Kumakamba[2], Christian Lange[3], Erby Obel[2], Guy Miningue[4], Jacques Likofata[5], Amethyst Gillis[6], Matthew LeBretton[7], David J. McIver[3], Jason Euren[6], Christine Kreuder Johnson[8], Tracy Goldstein[8], Jean J. Muyembe-Tamfum[4], Karen Saylors[9]

[1]*Labyrinth Global Health, Honolulu, HI, United States,* [2]*Metabiota Inc., Kinshasa, Democratic Republic of the Congo,* [3]*Metabiota Inc., Nanaimo, BC, Canada,* [4]*Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of the Congo,* [5]*Mbandaka Laboratory, Ministry of Health, Mbandaka, Democratic Republic of the Congo,* [6]*Metabiota Inc., San Francisco, CA, United States,* [7]*Mosaic, Yaoundé, Cameroon,* [8]*One Health Institute, School of Veterinary Medicine, University of California, Davis, Sacramento, CA, United States,* [9]*Labyrinth Global Health, Saint Petersburg, FL, United States*

Since Ebola virus disease (EVD) was first reported in 1976, six different species in the Ebolavirus genus have been discovered. Outbreaks were historically small and locally contained until the 2012-2014 West Africa epidemic which caused over 11,000 deaths. This outbreak spurred an international response, with a significant focus on both emergency health services and scientific research on understanding Ebolavirus transmission, spread, prevention, and the health system failures that lead to EVD propagation. Despite all efforts and lessons learned, EVD continues to be a problem, particularly in the Democratic Republic of the Congo (DRC) which is experiencing its 10th EVD outbreak, 9 of which have been caused by Zaire Ebolavirus (EBOV). In order to investigate perceptions of risk, exposure and beliefs and practices of high-risk bushmeat market workers, following the 2018 Ebola outbreak in Equateur Province, Democratic Republic of the Congo, we conducted behavioral interviews and collected serologic samples from bushmeat vendors and primates in Mbandaka to test for evidence of Ebola virus exposure. We asked questions about Ebola virus exposure, beliefs and experiences during the two reported outbreaks in Mbandaka. While participants indicated being aware of Ebola, they did not consider themselves at occupational risk for infection. We found antibodies against Zaire ebolavirus in one participant despite no reported history of disease or contact with infected individuals. Our data underline concerns of possible subclinical or undiagnosed Ebola virus infections and the importance and challenges of risk communication to populations who are occupationally exposed to bushmeat.

1114

CHLOROQUINE FOR THE TREATMENT OF *PLASMODIUM VIVAX* IN CENTRAL VIETNAM: *IN VIVO* THERAPEUTIC EFFICACY AND GENOMIC ANALYSIS OF PARASITE RECURRENCES

Eduard Rovira-Vallbona[1], Nguyen Van Hong[2], Johanna H. Kattenberg[1], Erin Sauve[1], Pieter Guetens[1], Ngoc Nguyen Thi Hong[2], Binh Nguyen Huong[2], **Anna Rosanas-Urgell**[1]

[1]*Institute of Tropical Medicine, Antwerp, Belgium,* [2]*NIMPE, Hanoi, Viet Nam*

Plasmodium vivax accounts for 40% of malaria cases in Vietnam, which occur mainly among ethnic minorities in forested areas. The national guidelines for the treatment of uncomplicated *P. vivax* infection recommends chloroquine (CQ) plus primaquine (PQ).A clinical trial evaluating CQ efficacy for *P. vivax* malaria was conducted in Gia Lai province between 2015 and 2016. Patients with fever and *P. vivax* mono infection were treated with 25 mg/kg of CQ over 3 days and PQ (0.25mg/kg/day for 14 days) at the end of 42 days follow-up. CQ and DCQ concentrations in blood were measured at day 7 and day of recurrence. Recurrences were genotyped using four microsatellites, a 38-SNP barcode (Spotmalaria), and whole genome sequencing (with pairwise comparisons by identity-by-descent (IBD)). *Ex vivo* susceptibility to CQ and other antimalarial drugs was determined by 48h schizont maturation assay. All samples were screened for SNPs in *pvmdr1* and expression levels of both *pvmdr1* and *pvcrt*. Out of 59 patients with completed 42-day follow-up, 18 (31%) had late recurrent *P. vivax* on days 35 (n=6) or 42 (n=12), all with CQ-levels below the MIC<100ng/ml. 13/15 recurrences were found to be homologous genotypes by microsatellite analysis, 6/11 using the SNP barcode and 9/14 had >98% of their genome IBD. Expression levels of *pvcrt* at day 0 were higher in patients with IBD-recurrences than those without recurrence or with non-homologous recurrences (p=0.0026). In addition, expression levels of both *pvcrt* and *pvmdr1* at the day of IBD-homologous-recurrence was higher than day 0 (p=0.019 and p=0.0039). This was not observed for non-homologous recurrences. Genotyping techniques revealed a high prevalence of homologous recurrences after CQ treatment in Central Vietnam. At the time of the study, PQ treatment was infrequent and intensity of transmission in Gia Lai one of the highest in Vietnam. Although all recurrences occurred on day 35 or later, recurrent parasites had increased levels of *pvcrt* expression, previously associated with CQ resistance.

1115

EVALUATING THE ANTIMALARIAL ACTIVITY OF MALARIA BOX COMPOUNDS AGAINST *PLASMODIUM FALCIPARUM*

Jersley Didewurah Chirawurah, Yaw Aniweh, Gordon Awandare

University of Ghana, Accra, Ghana

The emergence of drug-resistant malaria parasites to artemisinin and its combination therapies highlights the need to increase the arsenal of

new antimalarials with novel mechanisms of action. The Malaria Box compound library from the Medicines for Malaria Venture (MMV) meets this need by providing an accelerated path to the development of new, affordable and easily accessible antimalarial drugs for malaria-endemic regions. In our previous study, we identified MMV006087, MMV085203 and MMV008956 from the Malaria Box compound library, to have high potencies against clinical isolates of *Plasmodium falciparum*. To further validate the potency of these three compounds, we screened them against five laboratory and ten clinical isolates *P. falciparum* using optimized *in vitro* growth inhibitory assays. Additionally, we evaluated the response of two clinical isolates (EIMA375 and EIMA 377) to the three compounds over three months. On average, we found the laboratory strains (average IC_{50} of 147 nM) to be more susceptible to the three Malaria Box compounds compared to the clinical isolates (average IC_{50} of 362 nM). MMV006087 was the most potent compound with an average IC_{50} of 25.14 nM compared to MMV008956 (average IC_{50} of 232 nM) and MMV085203 (average IC_{50} of 507 nM). We also observed that the three compounds had decreased IC_{50} values against the two isolates; EIMA375 and EIMA 377, in growth inhibitory assays conducted at different time points within three months. The data from this study validate the potency of MMV006087 as a potential lead antimalaria drug candidate. The differences in the response of the laboratory and clinical isolates to the three Malaria Box compounds also substantiate the need to include clinical isolates during antimalarial compound screening programmes.

1116

EFFECTS OF ISOLATE REFRIGERATION AND DRUG STORAGE ON*EX VIVO* DRUG SENSITIVITIES OF *PLASMODIUM FALCIPARUM* FIELD SAMPLES FROM UGANDA

Martin Okitwi[1], Thomas Katairo[1], Patrick K. Tumwebaze[1], Stephen Orena[1], Oswald Byaruhanga[1], Samuel L. Nsobya[1], Brett R. Bayles[2], Melissa D. Conrad[3], Philip J. Rosenthal[3], Roland A. Cooper[2]

[1]*Infectious Disease Research Collaboration, Kampala, Uganda,* [2]*Dominican University of California, San Rafael, CA, United States,* [3]*University of California, San Francisco, CA, United States*

Malaria remains an overwhelming problem in Africa, where control is threatened by drug resistance. *Ex vivo* assays have been used to evaluate the sensitivity of *P. falciparum* to antimalarials. Short-term refrigerated storage of field samples facilitates surveillance at sites distant from the laboratory, but the effects of parasite storage on *ex vivo* assay results are unknown. We assessed 130 *P. falciparum* samples collected from malaria patients in Tororo and Busia in eastern Uganda, using a standard 72-h SYBR Green assay, to compare *ex vivo* sensitivities of isolates assayed on the same day of sample collection (day 0) and after overnight storage at 4-8˚C (day 1), against 8 antimalarials. We compared median 50% inhibitory concentrations (IC50s) on day 0 and on day 1 using the Wilcoxon matched-pairs signed rank test. The median time from sample collection to initiation of *ex vivo* assays was 4.9 h on day 0 and 23 h on day 1. IC50s differed only slightly between day 0 and day 1 for all tested drugs, with results mostly slightly lower on day 1: chloroquine (24 vs 22 nM, p=0.02), MDAQ (7.3 vs 6.8 nM, p=0.008), piperaquine (8.1 vs 7.2 nM, p=0.08), pyronaridine (1.6 vs 1.1 nM, p<0.0001), mefloquine (12 vs 9.9 nM, p=0.10), lumefantrine (5.8 vs 4.9 nM, p=0.0002), DHA (1.9 vs 1.3 nM, p<0.0001), and pyrimethamine (41,645 vs 43,055 nM, p=0.19). We also observed that parasite growth over 72 h in drug-free wells was reduced by 20% in day 1 assays relative to day 0 assays (p<0.0001). We have found that sensitivities against laboratory and field strains can vary significantly for certain drugs based on methods of drug dilution and storage, which likely effects limits on solubility and stability for many compounds. We note concerns for lumefantrine and mefloquine, for which unusually high IC50s can be seen if compound solubilization is inadequate before serial dilution or resulting from multiple freeze-thaws of DMSO stocks. In summary, preparation of diluted drug stocks up to 24 h before assay initiation had minimal effects on *ex vivo* IC_{50} determinations, but care to assure solubilization and limiting freeze-thaws is essential to obtaining reliable measures of *ex vivo* drug sensitivity.

1118

INCREASE IN FREQUENCY OF A NEW *PLASMODIUM FALCIPARUM PFDHFR/PFDHPS* SEXTUPLE MUTANT CONTAINING A NOVEL *DHPS* MUTATION AT CODON 436 IN WESTERN KENYA

M. Andreína Pacheco[1], Kristan A. Schneider[2], Qiuying Cheng[3], Elly O. Munde[4], Caroline Ndege[5], Clinton Onyango[5], Evans Raballah[6], Samuel B. Anyona[7], Collins Ouma[5], Douglas J. Perkins[3], Ananias A. Escalante[1]

[1]*Temple University, Philadelphia, PA, United States,* [2]*University of Applied Sciences Mittweida, Technikumplatz, Mittweida, Germany,* [3]*Center for Global Health, University of New Mexico Health Sciences Center, Albuquerque, NM, United States,* [4]*Department of Clinical Medicine, School of Health Sciences, Kirinyaga University, Kerugoya, Kenya,* [5]*Department of Biomedical Sciences and Technology, Maseno University, Maseno, Kenya,* [6]*School of Public Health, Biomedical Sciences and Technology, Masinde Muliro University of Science and Technology, Kakamega, Kenya,* [7]*Department of Medical Biochemistry, School of Medicine, Maseno University, Maseno, Kenya*

Sulfadoxine-pyrimethamine (SP) is the only antimalarial drug formulation approved for intermittent prophylaxis in pregnancy (IPTp). However, mutations in the *Plasmodium falciparum dhfr* (*Pfdhfr*) and *dhps* (*Pfdhps*) genes confer resistance to pyrimethamine and sulfadoxine, respectively. Here, the frequencies of SP resistance-associated mutations were compared in samples from Kenyan children with malaria residing in a holoendemic transmission region between three time periods: 2005 (n=81), 2010 (n=95), and 2017-2018 (n=98). Since artemisinin-based combination therapy (ACT) is the current first-line treatment for malaria, the presence of mutations in the propeller domain of *P. falciparum* Kelch13 gene (*Pfk13*) linked to ACT-delayed parasite clearance was studied in the 2017/18 samples. Among other changes, a novel point mutation of *Pfdhps* S436H increased in frequency from undetectable in 2005 to 28% in 2017/18. Triple *Pfdhfr* mutant allele increased in frequency from 84% in 2005 to 95% in 2017/18, while the frequency of *Pfdhfr* double mutant alleles declined (allele CICNI from 29% in 2005 to 6% in 2017/18, and CNRNI from 9% in 2005 to undetectable in 2010 and 2017/18). Thus, a multilocus *Pfdhfr/Pfdhps* sextuple mutant genotype containing the novel *Pfdhps* S436H mutation increased in frequency from 2010 to 2017/18. Although none of the mutations associated with ACT-delayed parasite clearance was observed, the *Pfk13* mutation A578S, the most widespread *Pfk13* SNP found in Africa, was detected in low frequency. Overall, the changes in SP mutant allele frequency are consistent with drug pressure even though the drug is no longer the first-line treatment against *P. falciparum* malaria in Kenya.

1120

ATOVAQUONE-PROGUANIL RESISTANCE IN AN IMPORTED FALCIPARUM MALARIA CASE IN CHILE

Stella M. Chenet[1], Alan Oyarce[2], Thomas Weitzel[3], Jorge Fernández[2], Juan R. Tejedo[1], Udhayakumar Venkatachalam[4], Maria I. Jercic[2], Naomi Lucchi[4]

[1]*UNTRM, Chachapoyas, Peru,* [2]*Instituto de Salud Publica de Chile, Santiago, Chile,* [3]*Facultad de Medicina Clínica Alemana. Universidad del Desarrollo, Santiago, Chile,* [4]*Centers for Disease Control and Prevention, Atlanta, GA, United States*

Chile is one of the South American countries certified as malaria free; however, the recent increase of imported malaria cases has led to a malaria surveillance system aimed at informing treatment policies of imported cases. The World Health Organization (WHO) recommended first-line treatment for *Plasmodium falciparum* in malaria-endemic countries is an artemisinin combination therapy (ACT); nevertheless, in non-endemic countries, such as Chile, atovaquone-proguanil (Malarone™) is often used. In 2018, a patient, who reported a trip to Nigeria, was diagnosed with malaria by the Chilean Institute of Public Health (ISP). The patient was treated with Malarone™ and monitored until symptoms reappeared

on day 29. We performed a real-time PCR for parasite quantification and genotyping analysis for drug-resistance markers in six samples collected on different days: day of treatment (D0), D9, D29, D34, D64 and D83. Results on D9 showed a decrease in parasite density, from 8000 parasites/µl on Day 0 to 10 parasites/ µl; however, on D29, parasite density increased to 3400 parasites/ µl. The patient was treated with mefloquine after which a decline in parasitemia was observed with resolution by D83. Sanger sequencing results of parasites collected on D29 and D34 showed the presence of the Tyr268Cys mutation in the *cytochrome b* gene, associated with atovaquone resistance (this mutation was not detected in day 0). The *kelch13* genotyping results showed no mutations associated with artemisinin resistance. In conclusion, close follow-up of antimalarial treatment response is key in non-endemic countries, as it allows for the detection of polymorphisms associated with drug resistance, which in turn helps ascertain the appropriateness of antimalarial treatment policies of imported cases and provides information about drug-resistant genotypes entering countries such as Chile.

1121

PROBING THE ROLE OF MFR4 IN HALOFUGINONE RESISTANCE AND THE ADAPTIVE PROLINE RESPONSE

Akansha Pant[1], Amy A. Deik[2], Lola Fabgami[1], Claudia R. Taccheri[1], Mark Tye[3], Selina E. Bopp[1], Clary B. Clish[2], Ralph Mazitschek[3], Amanda K. Lukens[1], Dyann F. Wirth[1]

[1]*Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health, Boston, MA, United States,* [2]*Metabolomics Platform, The Broad Institute, Cambridge, MA, United States,* [3]*Center for Systems Biology, Massachusetts General Hospital, Boston, MA, United States*

Halofuginone (HFG) is a potent antimalarial that targets the *P. falciparum* cytoplasmic prolyl tRNA synthetase (*Pf*cPRS). Previously, we showed that parasites selected for an extended time with increasing concentrations of HFG can develop high-level resistance conferred by mutations in the *Pf*cPRS target. In contrast, short-term exposure with a low dose of HFG yielded parasites with a moderate resistance profile yet no mutation in the *pfcprs* locus. Further investigation revealed that exposure to HFG triggers a novel mechanism of drug tolerance, termed the Adaptive Proline Response (APR), wherein intracellular proline levels increase twenty-fold. This metabolic adaptation persists after drug withdrawal and renders parasites resistant to HFG. Labeled metabolomic experiments identified the arginine-to-proline biosynthetic pathway as the predominant source of the elevated proline observed in HFG-resistant parasites. To dissect the role of this pathway in the APR, we used CRISPR/Cas9 genome editing to knock out the ornithine δ-aminotransferase enzyme (ΔOAT parasites). Interestingly, some ΔOAT parasites exposed to low levels of HFG demonstrated HFG-tolerance despite a complete absence of proline biosynthesis from arginine. Whole genome sequencing of HFG-tolerant ΔOAT parasites identified a number of mutations in the *mfr4* gene (PF3D7_0914700) likely resulting in gene loss of function. To understand the contribution of MFR4 to HFG-resistance, we used CRISPR/Cas9 to disrupt the locus and generate MFR4 knockout parasites (ΔMFR4). ΔMFR4 parasites showed a twenty-fold increase in HFG dose-response relative to wild type (Dd2 EC_{50} = 0.7nM, ΔMFR4 EC_{50} = 14nM). Metabolomic studies to understand the intracellular proline levels of ΔMFR4 parasites are ongoing. MFR4 belongs to the major facilitator superfamily of transporters which remain largely uncharacterized in *P. falciparum*. We hypothesize that *mfr4* could be a drug transporter leading to HFG resistance when disrupted. Future efforts aim to unravel the underlying mechanisms involving putative transporter proteins and exploit them for drug discovery.

1122

PFMFR3, A NOVEL DRUG RESISTANCE MITOCHONDRIAL TRANSPORTER IN *PLASMODIUM FALCIPARUM*

Frances Maureen Rocamora[1], Purva Gupta[1], Eva Istvan[2], Krittikorn Kumpornsin[3], Prianka Kumar[1], Miranda Song[1], Sabine Ottilie[1], Daniel E. Goldberg[2], Marcus C. Lee[3], Elizabeth A. Winzeler[1]

[1]*University of California San Diego, San Diego, CA, United States,* [2]*Washington University School of Medicine, Saint Louis, Saint Louis, MO, United States,* [3]*Wellcome Sanger Institute, Hinxton, United Kingdom*

While global control programs have made great strides toward the eradication of malaria, the constant threat of antimalarial resistance remains a significant obstacle to complete elimination. In this regard, *in vitro* models of drug resistance provide a useful platform for studying not only the mechanism of action of antimalarials, but also identifying molecular markers of resistance against clinically relevant and novel compounds. By generating resistance through *in vitro* evolution followed by whole genome analysis, we were able to glean insight into the mechanisms of action and resistance of two structurally related, candidate antimalarials that have activity against the liver-, blood- and sexual stages of the parasite: MMV085203 and GNF-Pf-3600. Whole genome sequencing of *in vitro*-derived, resistant parasite lines identified a spectrum of mutations converging in the orphan apicomplexan transporter PF3D7_0312500 (*pfmfr3*) belonging to the major facilitator superfamily (MFS). Likewise, *in vitro* selection of MMV085203 in a *S. cerevisiae* model also resulted in mutations in a MFS transporter of siderophore-iron chelates, *arn1*. Disruption of *pfmfr3* confirmed the importance of this transporter in mediating resistance to MMV085203 and GNF-Pf-3600, as well as resulted in a significant decrease in sensitivity to frontline antimalarial, atovaquone. Despite the structural similarity of both compounds to atovaquone, a dihydroorotate dehydrogenase rescue assay using transgenic parasite lines suggests a different mechanism of action for these diaminonaphthoquinones than direct inhibition of cytochrome bc1. GFP-tagging of PfMFR3 revealed that it localizes to the parasite mitochondrion. This work is the first to distinguish the involvement of this previously uncharacterized transporter in antimalarial drug resistance. And given that *pfmfr3* is naturally polymorphic and sits in a chromosomal region that shows evidence of recent positive selection in sub-Saharan Africa, it could also represent a reservoir of clinically relevant resistance genotypes in the future.

1123

PLASMODIUM FALCIPARUM PLASMEPS IN 2/3 AMPLIFICATIONS AUGMENT MUTANT PFCRT-DRIVEN PIPERAQUINE RESISTANCE

SATISH K. DHINGRA, Sachel Mok, Tomas Yeo, David A. Fidock

Columbia university, New York, NY, United States

Rapidly spreading piperaquine (PPQ) resistance in Southeast Asia poses a major threat to malaria control in this region. Recent reports of rapidly declining efficacy of a key artemisinin-based combination therapy, dihydroartemisinin + PPQ have prompted investigations into the molecular basis of resistance. Leveraging genome-wide association data of clinical isolates from Cambodia, two publications independently reported the amplification of *Plasmodium falciparum plasmepsin 2/3* (*Pfpm 2/3*) genes as the molecular marker of PPQ resistance. Studies from our lab, leveraging zinc-finger nuclease-mediated gene editing of the *Plasmodium falciparum* chloroquine resistance transporter (PfCRT) demonstrated that several Cambodian PfCRT mutations (F145I, M343L and G353V) engineered into a laboratory Dd2 strain can confer high-grade PPQ resistance without *pfpm 2/3* amplification. Recent work has now provided compelling evidence that the rapidly emerging region-specific PfCRT mutations T93S, H97Y, F145I and I218F have become predominant mediators of PPQ resistance in Southeast Asia. These PfCRT mutations have emerged on a specific parasite KEL1/PLA1 co-lineage, which carries the PfK13 C580Y variant (KEL1) on an amplified *pfpm 2/3* genetic

background (PLA1). This continued evolution of novel *pfcrt* variants in the presence of multicopy *pfpm 2/3* invokes an important role for these amplifications in mutant *pfcrt*-driven PPQ resistance in field isolates. Using isogenic clones from two contemporary Cambodian isolates of the KEL1/PLA1 co-lineage that are mutant *pfcrt* and express different copies of *pfpm 2/3*, we provide conclusive evidence that amplification of *pfpm 2/3* augmented the survival of parasites to PPQ. We observed a 1.3-3.0-fold increase in parasite survival across a range of PPQ concentrations, in a PPQ survival assay. Ongoing work aims to replace the mutant *pfcrt* alleles in these isogenic clones with the Southeast Asian Dd2 allele to assess the role of *pfpm 2/3* amplifications on a wild-type *pfcrt* background and to understand the driving factors behind their selection.

1124

PRIMAQUINE PHARMACOGENOMICS: IDENTIFICATION OF HUMAN URINARY METABOLITES ASSOCIATED WITH CYTOCHROME P450 2D6

Babu L. Tekwani[1], Yanhong Wang[2], Washim Khan[2], Bharathi Avula[2], NP Dhammika Nanayakkara[2], HTM Bandera Herath[2], Narayan D. Chaurasiya[1], Andrea Gaedigk[3], Rajeev Mehlotra[4], Peter A. Zimmerman[4], **Larry A. Walker**[2]

[1]*Southern Research, Birmingham, AL, United States,* [2]*National Center for Natural Products Research, University of Mississippi, University, MS, United States,* [3]*Division of Clinical Pharmacology, Children's Mercy Kansas City, Kansas City, MO, United States,* [4]*The Center for Global Health and Disease, Case Western Reserve University, Cleveland, OH, United States*

The efficacy of primaquine (PQ) is regarded to be dependent upon biotransformation via cytochrome P450 2D6 (CYP2D6), and it is postulated that 5-hydroxyprimaquine is the key active metabolite. However, PQ metabolism by CYP2D6 is complex, and there are multiple additional pathways, both CYP and non-CYP-mediated. In the course of clinical studies of PQ metabolism and pharmacokinetics, we identified one subject out of 7 (Subj B) with a *CYP2D6*4/*4* genotype (Activity Score, AS=0, predicting poor metabolizer status). The other 6 subjects had genotypes predicting intermediate (AS=1) or normal (AS=1.5 or 2.0) metabolism. Analyses of plasma and urinary metabolites of PQ were done by LC/MS and MS/MS. PQ plasma concentration rose more rapidly and achieved higher levels in Subj B than in the other subjects. The plasma concentration of carboxyPQ, derived via monoamine oxidase, was not changed in comparison to the other subjects. Among five other plasma metabolites identified, none were absent or markedly altered in Subject B. However, urinary metabolite excretion showed >20 putative metabolites in the urine of all 7 subjects post-dosing, but absent in pre-dose urine collections. Seven additional metabolites were present in all of the other 6 subjects, but not Subject B. These consisted of: 4 hydroxylated PQ metabolites, [2-OH-PQ, 3-OH-PQ, 4-OH-PQ, and 5-OH-PQ, all *m/z* 276]; PQ-5,6-o-quinone (*m/z* 260); 6-demethyl-PQ glucuronide (*m/z* 422); and a metabolite (*m/z* 494) hydroxylated and glucuronidated on the quinoline ring, and acetylated on the terminal amine. The 5-OH-PQ assignment is tentative, due to lack of a stable synthetic standard. But the o-quinone confirms this route. The studies confirm our *in vitro* findings with recombinant human CYP2D6, indicating PQ hydroxylation at positions 2,3,4, with the *o*-quinone reflecting 5-OH-PQ; they also suggest the presence of 5-OH-PQ in urine, and provide evidence for 6-demethylation, and for phase II conjugates of CYP2D6 products. Further studies are needed to quantify these metabolites, ascertain the relevance of each to PQ efficacy, and define the contribution of genetic variation on PQ metabolite patterns.

1125

CONTINUED IMPROVEMENTS IN THE ACCURACY OF ANTIMALARIAL DRUG LEVEL MEASUREMENTS PERFORMED WORLDWIDE: RESULTS FROM 10 YEARS OF PROFICIENCY TESTING CONDUCTED BY WWARN

Ranitha Vongpromek[1], Christiaan Lourens[1], Pak Sadomthian[1], Teeradet Khomvarn[1], Philippe J. Guérin[2], Joel Tarning[3], **Mehul Dhorda**[3]

[1]*Infectious Diseases Data Observatory (IDDO) & WorldWide Antimalarial Resistance Network (WWARN), Asia-Pacific Regional Centre, Bangkok, Thailand,* [2]*Infectious Diseases Data Observatory (IDDO) & WorldWide Antimalarial Resistance Network (WWARN), Oxford, Oxford, United Kingdom,* [3]*Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand*

Accurate drug level measurements are crucial for optimization of antimalarial dosing and confirmation of emerging resistance. To demonstrate accuracy and comparability of such measurements between laboratories and over time, laboratory performance must be independently verified. We present results from 9 rounds of proficiency testing conducted over 10 years by the ISO17043-accredited WWARN Proficiency Testing (WWARN-PT) scheme for antimalarial drug level measurements. Each round of PT consisted of 2 cycles; laboratories received 12 human plasma samples per cycle, spiked with varying amounts of antimalarials, and measured levels of each drug using in-house validated methods. Their performance was evaluated by calculating and grading the Z-score, where |Z-score|<2 was considered 'satisfactory', 2≥|Z-score|>3 'questionable' and |Z-score|≥3 'unsatisfactory'. Z-scores were transformed to their natural logarithms to obtain a normal distribution and subsequently fitted with a log-linear model for analysis. From 2010-2019, 11 participating laboratories reported 6,315 results for 14 antimalarial drugs. Z-scores ranged from <0.001 to 210.72 of which 93.2% were graded 'satisfactory', 3.2% 'questionable' and 3.6% 'unsatisfactory'. In 7 laboratories which participated in ≥3 rounds of PT, the proportion of Z-scores <1 increased from 50.3% to 85.5% between the first and last year of participation. Ln(Z-scores) decreased in 5/7 of these laboratories with the reduction being statistically significant for 2 laboratories. The lowest mean Z-score and the highest proportion of satisfactory results were attained after a median of 6 rounds (range: 2-7) and 7 rounds (range: 1-8) of PT, respectively. Laboratories participating in the WWARN-PT scheme showed sustained improvements in the accuracy of antimalarial drug level measurements. Participating in the WWARN-PT confirmed and/or helped to enhance accuracy of participants' measurements, thus increasing the value and reliability of their results generated to support improvements in antimalarial treatments.

1126

VARIED SENSITIVITIES TO NOVEL MMV PIPELINE ANTIMALARIALS OF UGANDAN *PLASMODIUM FALCIPARUM* ISOLATES AND ASSOCIATIONS WITH SPECIFIC GENOTYPES

Oriana Kreutzfeld[1], Stephanie A. Rasmussen[2], Patrick Tumwebaze[3], Oswald Byaruhanga[3], Thomas Katairo[3], Martin Okitwi[3], Stephen Orena[3], Jenny Legac[1], Melissa Conrad[1], Samuel Nsobya[3], Ozkan Aydemir[4], Jeff Bailey[4], Maelle Duffey[5], Brett Bayles[2], Philip J. Rosenthal[1], Roland Cooper[2]

[1]*UCSF, San Francisco, CA, United States,* [2]*Dominican University, San Rafael, CA, United States,* [3]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [4]*Brown, Providence, RI, United States,* [5]*Medicines for Malaria Venture, Geneva, Switzerland*

Among novel compounds under investigation as potential antimalarials are inhibitors of PfATP4, PfDHODH, PfEF2, PfCYTB, PfDHFR and compounds with resistance reportedly mediated by variation in PfCARL. We assessed *ex vivo* sensitivities to these compounds of 561 fresh *Plasmodium falciparum* isolates from Tororo and Busia districts in Uganda from 2016-2019. We then characterized genotypes of parasites with varied sensitivity to these antimalarials. We observed varied degrees of sensitivities

of Ugandan isolates to all tested compounds. For inhibitors of PfEF2 (DDD498) and PfDHODH (DSM265, DSM421, DSM632, DSM705, and MMV258), sequencing of target genes demonstrated no mutations clearly associated with altered sensitivities. For the PfCYTb inhibitor ELQ300, a A205V mutation located close to the cytochrome b inhibitor-binding site in a single isolate was associated with an unusually high IC_{50}. For the PfDHFR inhibitor P218, the I164L mutation was associated with decreased sensitivity (mean IC_{50} 1 nM for WT, 5 nM for mutant, $p<0.05$). Previous studies showed that occurrence of I164L mutation increased levels of resistance to other antifolates. For KAF156 the Y353C (mean IC_{50} 36 nM for WT, 75 nM for mixed) and Q605L (mean IC_{50} 27 nM for WT, 94 nM for mixed) mutations in the resistance mediator PfCARL were associated with decreased sensitivity. For the three PfATP4 inhibitors mean IC_{50}s were 57 nM for SJ733, 9 nM for PA92, and 0.4 nM for KAE609. A G223S mutation was associated with decreased sensitivity to SJ733 (mean IC_{50} 71 nM for WT, 93 nM for mutant), PA92 (IC_{50} 10 nM for WT, 15 nM for mutant) and KAE609 (IC_{50} 0.5 nM for WT, 0.7 nM for mutant); for all comparisons $p<0.05$. Interestingly, previous studies showed that incubation of a laboratory strain with an analog of KAE609 selected for resistant parasites with a mutation (G223R) at the same codon. Our results indicate that malaria parasites circulating in Uganda have a number of polymorphisms associated with varied sensitivity to MMV pipeline compounds, although the clinical relevance of the differences in sensitivity of field isolates is uncertain.

1127

RING-STAGE SURVIVAL AND *IN VITRO* FITNESS OF *PLASMODIUM FALCIPARUM* KELCH13 MUTANTS

Barbara H. Stokes[1], Satish K. Dhingra[1], Ioanna Deni[1], Kelly Rubiano[1], Jade R. Bath[1], Josefine U. Striepen[1], Nina F. Gnädig-Schoenfeld[1], Sachel Mok[1], Philip J. Rosenthal[2], Didier Ménard[3], David A. Fidock[1]

[1]*Columbia University, New York, NY, United States,* [2]*UCSF, San Francisco, CA, United States,* [3]*Institut Pasteur, Paris, France*

The emergence of artemisinin (ART) resistance in intraerythrocytic *Plasmodium falciparum* ring-stage parasites has led to increased rates of treatment failure with first-line ART-based combination therapies in Southeast Asia. Select mutations in K13 result in decreased parasite susceptibility in clinical settings and enhanced parasite survival in the ring-stage survival assay (RSA) conducted with early post-invasion parasites *in vitro*. The most important of these is the K13 C580Y mutation that has reached fixation in Cambodia, the epicenter of ART resistance in the Greater Mekong Subregion (GMS), and that has been validated as a driver of ART resistance by gene-editing experiments. Nonetheless, several reports have described additional, uncharacterized K13 polymorphisms both within and outside of the GMS. Within the GMS, these include the E252Q, F446I, P553L, R561H and P574L mutations. Outside of the GMS, of particular interest are the R561H mutation, recently detected in Rwanda; the M579I, R622I and A675V mutations, reported in small numbers and associated with delayed parasite clearance in Ethiopia, Uganda, and Equatorial Guinea, respectively; and the G625R mutation, associated with delayed parasite clearance in India. Here we report CRISPR/Cas9-mediated editing and RSA phenotyping of these mutations in distinct parasite genetic backgrounds, including reference strains and recent Southeast Asian and African isolates. Using the C580Y mutation as a reference, we demonstrate that K13 mutations can indeed confer ART resistance in parasites of African origin, but that the degree of resistance varies significantly depending on the mutation and strain background. We also provide evidence that reduced fitness of K13 mutants may provide a counter-selective pressure against the spread of resistance-conferring alleles. Lastly, we test the contribution of polymorphisms in two proposed secondary determinants of ART resistance, including the D193Y mutation in the apicoplast protein ferredoxin and the T484I mutation in *P. falciparum* multidrug-resistance protein 2, and find that these are not able to modulate ART resistance *in vitro*.

1127A

WITHIN-INFECTION PARASITE COMPLEXITY IS ASSOCIATED WITH DIFFERENTIAL CLEARANCE RATES OF *PLASMODIUM FALCIPARUM* IN KENYA AND TANZANIA

Kara A. Moser[1], Billy Ngasala[2], Lwidiko E. Mhamilawa[2], Peter O. Oluoch[3], Oksana Kharabora[1], Molly Deutch-Feldman[4], Andreas Mårtensson[5], Stephen R. Meshnick[4], Ann Moormann[6], Madeline Denton[1], Ozkan Aydemir[7], Nicole Mideo[8], Boaz Odwar[3], Jeffrey A. Bailey[7], Hoseah Akala[3], John Michael Ong'echa[3], Jonathan J. Juliano[1]

[1]*Institute for Global Health and Infectious Diseases, University of North Carolina Chapel Hill, Chapel Hill, NC, United States,* [2]*Muhimbili University of Health and Allied Sciences, Dar es Salam, United Republic of Tanzania,* [3]*Center for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya,* [4]*Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina Chapel Hill, Chapel Hill, NC, United States,* [5]*Department of Women's and Children's Health, International Maternal and Child Health, Uppsala University, Uppsala, Sweden,* [6]*Department of Medicine, University of Massachusetts Medical School, Worcester, MA, United States,* [7]*Department of Pathology and Laboratory Medicine, Brown University, Providence, RI, United States,* [8]*Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, ON, Canada*

Resistance to artemisinin combination therapies (ACTs) remains a major threat to malaria control world-wide. While most clinical trials of uncomplicated *Plasmodium falciparum* malaria report ACT efficacy >95% in Africa, there have been reports describing decreasing efficacy for both clinical failure by day 28 and prolonged parasite clearance rates. Parasite clearance can be impacted by host and parasite factors, and there may be variation in clearance rates from *P. falciparum* infections in Africa. A better understanding of such variation, and risk factors associated with relatively slow clearance rates, is important for understanding clinical responses to ACTs in Africa. To this end, 300 children aged 2-10 years with acute uncomplicated *P. falciparum* infections were enrolled from 2016-2018 at two sites (Ahero, Kenya and Bagamoyo District, Tanzania) after receiving treatment with artemether-lumefantrine. Blood samples were taken at 0, 24, 48, and 72 hours, and weekly until 28 days after treatment. Parasite and host genetics were assessed, as well as clinical, behavioral, environmental, and host anti-malarial serologic responses characteristics. While there was a range of clearance rates (as measured by parasite slope half-life) in both study sites, 79% and 90% of Kenyan and Tanzanian samples, respectively, had detectable parasites at 72 hours post treatment by real-time PCR. No patients were microscopically positive at 72 hours. Recrudescence events (n=9) were also observed after treatment using msp1/msp2 genotyping; the majority of these infections were monoclonal at all time points (as measured by AMA1 amplicon deep sequencing). While most host characteristics were not associated with slower parasite clearance rates, the AMA1 deep amplicon sequencing suggested an association between increased complexity of infection at baseline and faster clearance rates. The study provides important data on how *P. falciparum* populations in East Africa respond to ACT, and how complexity of infection may affect clearance rates.

1128

LAMP ASSISTED RAPID AND ULTRASENSITIVE MOLECULAR DIAGNOSTIC TOOL FOR HIGH-THROUGHPUT SCREENING OF MALARIA

Mingzhu Yang, Hongru Pian, Zhi Zheng

Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing, China

As a serious life-threatening disease, malaria cause more than 400 000 deaths every year. The control and elimination of malaria is still a global challenge. Accurate diagnosis and timely treatment of malaria infections is the key to the control of malaria. Traditional microscopy and rapid diagnostic test offer poor sensitivity, which can hardly identify

asymptomatic carriers. Quantitative PCR can provide highly sensitive results, but rely on time-consuming procedures and expensive instruments. A sensitive, rapid, simple and affordable detection method is urgently required for the diagnosis of malaria. We develop a capture and ligation-assisted loop-mediated isothermal amplification (CLIP-LAMP) strategy for the rapid and ultrasensitive detection of *Plasmodium*. 18S rRNA of *Plasmodium* is released after cell lysis and hybridized with the ligation probe, as well as the capture probe modified on the surface of magnetic particles. Product of the sandwich hybridization is separated and enriched under the external magnetic field. Double stranded template is formed after ligation for the isothermal amplification. We perform the test of parasite diluted with whole blood lysate to evaluate the sensitivity of the method. To further investigate the performance of the method in screening of malaria, we detect dried blood spots of volunteers and compare the results with standard detection methods. CLIP-LAMP shows a wide linear range from 100 to 0.001 p μL^{-1} with a detection limit of 0.0004 p μL^{-1}. The whole detection process can be completed within 90 minutes. In the test of dried blood spots, all the results are consistent with standard quantitative RT-PCR. Microscopy identified 3 of the 11 positive samples, while rapid diagnostic test identified only 1 positive sample. CLIP-LAMP eliminates the reliance of complicated procedure for nucleic acid extraction and reverse transcription, as well as sophisticated instruments. The high sensitivity, rapidity, simplicity, and cost-effectiveness enable CLIP-LAMP a promising approach to perform the active molecular screening for the elimination of malaria in low-resource regions.

1129

DEVELOPMENT OF A RAPID DIAGNOSIS TEST SPECIFIC FOR *PLASMODIUM VIVAX*

Karli R. Redinger, Quentin D. Watson, Marlin Linger, Rajeev K. Mehlotra, Peter A. Zimmerman, Jürgen Bosch

Case Western Reserve University, Cleveland, OH, United States

One of the main obstacles in properly diagnosing and treating individuals with *Plasmodium vivax* (*Pv*) malaria is that there is currently no diagnostic test that is specific to *Pv* antigens. Detection of *Pv* infection is crucial to preventing further transmission of Malaria disease because additional treatment is required to eradicate dormant *Pv* hypnozoites present in the liver which can cause blood stage infection decades after initial infection. In the Bosch Lab, we have selected, recombinantly expressed and refolded five *Pv*-specific proteins to serve as antigens for the production of antibodies to be used in the development of a Rapid Diagnosis Test (RDT) for *Pv*. The five protein targets of interest (*Pv*EBP2, *Pv*LDH, *Pv*DBP, *Pv*s25, and *Pv*EBP2-H7) were selected due to their specificity for *Pv* parasites as opposed to other *Plasmodium* species. These protein targets were generated in the lab and refolded using self-assembled protein nanoparticles (SAPNs) in order to produce a stabilized protein core with our antigens of interest exposed on the surface of the particle. A total of ten rabbits were immunized with the antigens to produce antibodies against the five protein targets. Subsequent testing in the lab determined that the antibodies generated were specific to the *Pv* antigens selected and did not react to uninfected red blood cell samples. Multiple detection methods have been used in the lab to investigate the detection limit of these antibodies as well as the cross-reactivity with *P. falciparum* (*Pf*) parasite proteins. Western blot assays have shown minimal cross-reactivity when *Pf*-infected cultures when probed against the five antibodies. Similar results were generated when mixed *Pv* and *Pf* infection samples were probed with the antibodies using both ELISA and paper-based assays thus indicating a high selectivity for *P. vivax* proteins as opposed to *Pf* proteins. Additionally, our initial experiments from ELISA-based assays indicate that four of the five antibodies have a detection limit below one *Pv* parasite/μL of blood. This detection limit is crucial for effectively diagnosing *Pv* infections in the field.

1130

IMPROVED MALARIA HEALTH PRODUCTS ACCOUNTABILITY THROUGH INTRODUCTION OF A LOGISTICS MANAGEMENT INFORMATION TOOL: A CASE STUDY OF A PRIMARY HEALTHCARE CENTER, KOJOLI MATERNITY HOME IN ADAMAWA, NIGERIA

Melis Esi[1], Isaac Adejo[1], Stephanie Xueref[2], Tom Hall[2], Mohammed Abdullahi[1], Olumide Elegbe[1], Beauty Okologo[3], Kenji Goyit[3], Victor Adebayo[4], Ezinne Onuba[4], Stanley Chidove[5]

[1]*Management Sciences for Health (MSH), Abuja, Nigeria,* [2]*Management Sciences for Health (MSH), Arlington, VA, United States,* [3]*National Malaria Elimination Program (NMEP), Abuja, Nigeria,* [4]*Catholic Relief Services (CRS), Abuja, Nigeria,* [5]*The Global Fund, Geneva, Switzerland*

Ensuring accountability for malaria health products (MHP) is a major challenge to the Nigeria health sector. Health workers record patient information and treatment prescribed in the outpatient register (OPR), while at the dispensing unit, MHP consumption is recorded using the inventory control card (ICC), a stock record keeping tool that does not track patient details. The lack of transparency on MHP consumption fosters gaps in accountability, which the wide discrepancy between service data and consumption data illustrates. To address this issue, Management Sciences for Health, in collaboration with the National Malaria Elimination Program and Catholic Relief Services, with support from the Global Fund Malaria Grant (2018-20) developed a new Logistics management information tool called a daily consumption register (DCR), which replaced the ICC used at the dispensing unit. The DCR allows tracing MHP dispensed to each individual patient, and when compared with the OPR, allows the health facility (HF) to check if both registers are in alignment. We examined the impact of this tool on one HF, the Kojoli Maternity Home (KMH) in Adamawa State, comparing data from September 2019 to February 2020. Before use of the DCR in November 2019, we observed a large variance between bi-monthly consumption data from the ICC and service data from the OPR with 150 rapid diagnostic tests, 191 artemisinin-based combination treatments (ACTs) and 50 long-lasting insecticidal nets (LLINs) consumed versus respectively 423 (65% variance) patients tested, 358 (47%) malaria cases confirmed and 243 (79%) pregnant women eligible to receive LLINs. Two dispensers were trained on MHP logistics management and the DCR and mentored during facility supervision visits over the next four months. An analysis performed at the end of February 2020 found 0% variance between consumption and service data for all products except a variance of 1% between ACTs and malaria cases. The result demonstrates that having a proper tool to track MHP consumed, coupled with training and supervision, can drive improved accountability at both the dispensing and outpatient service delivery units.

1131

HIGH-SENSITIVITY MALARIA DIAGNOSIS OF FEBRILE PATIENTS AT THE POINT-OF-CARE: INSIGHTS FROM A HIGH BURDEN CLINIC IN MALAWI

Rosalind E. Howes[1], Jullita Malava[2], Louis Banda[2], Camille Escadafal[1], Sabine Dittrich[1], Xavier C. Ding[1]

[1]*Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland,* [2]*Malawi Epidemiology and Intervention Research Unit (MEIRU), Chilumba, Malawi*

Diagnostic innovation is critical to better detect challenging malaria infections, such as the low density parasitaemias which are known to contribute to onward transmission in areas nearing elimination. An objective of the thorough evaluations of new tools is to ensure that they do not carry unintended consequences. The recently commercialised high-sensitivity rapid diagnostic tests (hsRDT) for *Plasmodium falciparum* are intended for active case detection of asymptomatic individuals within remaining pockets of transmission. There are concerns that their "off-label" use for the clinical case management of febrile patients could lead to misdiagnoses of other causes of the febrile episode as malaria due to the identification of low-density *P. falciparum* infections

or persistent PfHRP2/3 antigen detection. This could lead to a delay in patients accessing appropriate care in a timely manner. Previous studies have assessed this in low-endemic areas, here we consider a high-transmission setting where the absolute impact of the issue could be greater. Retrospective analysis of banked whole blood samples from 511 non-severe febrile adults and children seeking treatment at a rural clinic in Karonga district, Malawi, in 2017-2018 was conducted. Samples were tested by the standard of care RDT (Abbott 05FK60), high-sensitivity RDT (Abbott 05FK140) and molecular diagnosis of parasite mitochondrial DNA amplified by nested PCR (nPCR). There was 99.8% concordance across the RDTs, with a positivity rate of 48.1% by conventional RDT and 47.9% by hsRDT; a single sample was positive by routine RDT only, and confirmed by molecular diagnosis. For both RDTs, their sensitivity and specificity relative to nPCR was 68% and 97%, respectively. Molecular diagnosis found evidence of *P. malariae* and *P. ovale* (1.9% of samples). No *P. vivax* was detected. In conclusion, the hsRDT did not diagnose any patients as malaria positive who were not also diagnosed by the routinely used RDT. Use of the hsRDT would not therefore have altered the diagnosis and treatment of febrile patients at this clinic. These results corroborate findings from lower transmission settings.

1132

ACTIVE CASE DETECTION OF ASYMPTOMATIC MALARIA INFECTION IN PREGNANCY WITH LAMP: A PILOT RANDOMIZED INTERVENTIONAL STUDY IN ETHIOPIA

Guluma Tadesse[1], Claire Kamaliddin[2], Cody Doolan[2], Ranmalee Amarasekara[2], James Cheaveau[2], Ruth Legese[2], **Delenasaw Yewhalaw**[1], Dylan R. Pillai[2]

[1]*Jimma University, Jimma, Ethiopia,* [2]*University of Calgary, Calgary, AB, Canada*

Malaria in Pregnancy (MiP) is the direct consequence of *Plasmodium* infection during pregnancy. The sequestration of parasites in the placenta restricts fetal development and contributes to low birth weight (LBW) and anemia. In Ethiopia, the Ministry of Health recommends diagnosis of symptomatic cases of malaria using microscopy and/or rapid diagnostic tests. Since MiP is typified by low-level parasitemia, the sensitivity of RDT and microscopy may have limitations to detect MiP. We aimed to evaluate the impact of systematic active case detection of malaria using Loop mediated isothermal Amplification (LAMP) compared to microscopy. We conducted a longitudinal study in two health centers of Kafa zone, South West Ethiopia. One hundred-ninety nine women in the first or second trimester of pregnancy were enrolled in the study and randomized to either standard microscopy group (1/4 of women) or the LAMP intervention group (3/4 of women). Women were followed during pregnancy for a total of three antenatal care visits and delivery. Women testing positive for *Plasmodium* by any method were treated according to parasite species and gestational age as per Ethiopian guidelines. Haemoglobin level was measured in all women at each ANC visit and delivery. Newborn birth weight was recorded at delivery. Average gestational age at inclusion was 16.4 (+/- 5.8) weeks. 29.1% of included women were primiparous and 70.9% were multiparous. Socio demographic characteristics were similar between the two study arms. MiP prevalence was 2.1% in the LAMP arm and 0.7% in the microscopy arm. The proportion of LBW was higher in the microscopy (14 %, n = 7/50) than in the LAMP group (0%, n= 0/143) ($p = 4.10^{-5}$). The prevalence of anemia between the intervention group and the standard of care group was similar ($p = 0.567$). However, at the individual level, women treated for malaria displayed an average increase of 0.9 g of hemoglobin between intra-partum malaria diagnosis and delivery. Overall, our study demonstrated that LAMP can be implemented in health facilities for pregnant women attending antenatal care (ANC) and may reduce the clinical burden of MiP.

1133

INTEGRATION OF MALARIA SERVICES AT HIV CARE AND TREATMENT CLINICS (CTC) TO ENHANCE SERVICES FOR BETTER CLIENT OUTCOMES: EXPERIENCE FROM TWO HIGH MALARIA BURDEN REGIONS IN TANZANIA, 2019

Michael Gulaka[1], Jackson Mugyabuso[1], Zahra Mkomwa[1], Goodluck Tesha[1], Joackim Nyarigo[1], Emmanuel Lesilwa[1], Chonge Kitojo[2], Erik Reaves[3], Lazaro Samwel[4], Ally Mohamed[4], Abdallah Lusasi[4], Rita Noronha[5], Lusekelo Njonge[5], Lolade Oseni[6], Gladys Tetteh[6], Bill Brieger[6], Edward Kenyi[6], Benedicto Ngaiza[7], Francisca Charles[7], David Mndaba[7], Assessanda Kaniki[8], Julian Mugengi[8]

[1]*PATH Tanzania, Dar es Salaam, United Republic of Tanzania,* [2]*USAID/US President's Malaria Initiative, Dar es Salaam, United Republic of Tanzania,* [3]*CDC/US President's Malaria Initiative, Dar es Salaam, United Republic of Tanzania,* [4]*National Malaria Control Program, Dar es Salaam, United Republic of Tanzania,* [5]*Jhpiego, an affiliate of John Hopkins University, Dar es Salaam, United Republic of Tanzania,* [6]*Jhpiego, an affiliate of John Hopkins University, Baltimore, MD, United States,* [7]*Chato Health Management Team, Dar es Salaam, United Republic of Tanzania,* [8]*Kagera Regional Health Management Team, Dar es Salaam, United Republic of Tanzania*

Malaria and HIV cause more than 2 million deaths globally, and malaria cases in Tanzania are estimated to be 7 million per year. While WHO recommends integration of malaria and HIV services, People Living with HIV (PLHIV) suspected to have malaria are referred from HIV care and treatment centers (CTC) to a separate outpatient department for malaria testing and treatment. This requires patient time at two different clinics on the same day, which can delay receiving malaria services. Between January and December 2019, malaria testing and treatment services were integrated at CTCs under one roof. On-the-job training on malaria case management was conducted for 187 health workers at 44 CTC sites with a large number of clients from Geita and Kagera, high malaria burden regions. All PLHIV were screened for fever at the time of CTC attendance, defined as elevated temperature or history of fever in past 48 hours. Those with fever were tested at CTCs using a malaria rapid diagnostic test (mRDT). Uncomplicated malaria cases were treated with an artemisinin-based combination therapy (ACT) at the CTC while those with severe malaria were admitted or referred if the facility did not have inpatient services. Among 57,170 PLHIV attending CTC between January and December 2019, 50,912 PLHIV (89%) were screened for fever; of those screened, 499 (0.9%) had fever and were tested by mRDT. Overall 114 (23%) had a positive mRDT, of which 113 (99%) were uncomplicated malaria cases, while one patient had severe malaria. The patients with uncomplicated malaria were treated with ACT and the one with severe malaria was treated with injection artesunate before referral. Integration of malaria services into CTCs provided an opportunity for PLHIV attending CTC to avoid the delay in testing and treatment for malaria without transferring to different clinics on the same day. The data from this initiative will help the National Malaria and AIDS Control Programs to plan and scale-up the integration of HIV and malaria services at CTCs s to ensure comprehensive and life-saving patient care.

1134

ASSESSING THE USE OF ROUTINE ANTENATAL CARE CLINIC MALARIA TEST-POSITIVITY RATES AS ESTIMATES OF MALARIA BURDEN

Jordan Smith[1], Norberto Bosepa Cuba Cuba[1], Olivier Tresor Donfack Sontsa[1], Gninoussa Akadiri[1], Godwin Fuseini[1], Wonder P. Phiri[1], Jose Osa Osa Nfumu[2], Matilde Riloha Rivas[2], Carlos A. Guerra[3], Guillermo A. Garcia[3]

[1]*MCDI, Malabo, Equatorial Guinea,* [2]*Ministry of Health and Social Welfare, Malabo, Equatorial Guinea,* [3]*MCDI, Silver Spring, MD, United States*

Pregnant women attending antenatal care clinics (ANCs) have been proposed to serve as a pragmatic sentinel population for malaria surveillance in order to provide timely estimates of malaria prevalence,

rapidly inform control strategies, and measure progress. Multiple studies have suggested that the patterns of malaria prevalence among pregnant women attending ANCs are correlated with malaria patterns in their community. However, evidence on the representativeness of routine ANC malaria test-positivity and its relationship with prevalence in other population subgroups is still lacking. We aim to compare malaria test-positivity during antenatal care to annual population-based prevalence surveys to assess their potential in measuring malaria trends and progress towards elimination. Malaria test-positivity data from pregnant women attending their first ANC visit in public health facilities within all four districts of Bioko Island between January 2015 and January 2020 were compared to prevalence data from island-wide, all-age annual malaria indicator surveys carried out from August to September for the years 2015 to 2019. Multiple linear regression will be used to describe the difference between malaria test-positivity in pregnant women and respective comparison groups as a function of ANC test-positivity and potential covariates. This analysis will test if the relationship between ANC test-positivity and survey prevalence in population subgroups follows spatially, temporally, and biologically meaningful patterns. Pregnant women attending ANC are a pragmatic sentinel population to assess heterogeneity and trends in malaria prevalence in Bioko. Nevertheless, because ANC malaria test-positivity cannot be used to directly predict the prevalence in other population subgroups, complementary community-level assessments continue to be important. Further analyses should consider the impact of higher resolution spatial information, socioeconomic characteristics, HIV infection, gestational age, and parasite densities on these correlations.

1135

KINETICS OF HRP-2 DETECTION BY ALERE™ ULTRASENSITIVE MALARIA AG *PLASMODIUM FALCIPARUM* RAPID DIAGNOSTIC TEST AFTER ARTEMETHER-LUMEFANTRINE TREATMENT IN CHILDREN IN BUSIA, UGANDA

Justin Goodwin[1], Azan Z. Virji[2], Richard Kajubi[3], Sarah Lapidus[2], Moses Were[3], Meghan Whalen[4], Francesca T. Aweeka[4], Norah Mwebaza[3], Sunil Parikh[1]

[1]*Yale School of Medicine, New Haven, CT, United States,* [2]*Yale School of Public Health, New Haven, CT, United States,* [3]*Infectious Disease Research Collaboration, Kampala, Uganda,* [4]*University of California San Francisco, San Francisco, CA, United States*

Malaria rapid diagnostic tests (RDTs) are now widely used for field-deployable or clinical diagnosis, particularly in areas with limited microscopy. Antigens detected by RDTs can persist in the blood after initial parasite clearance, potentially complicating efforts to diagnose subsequent infections and interpret malaria prevalence surveys. While more sensitive RDTs will improve our ability to detect the reservoir of infection, the duration of positivity following treatment may be more problematic. In particular, the kinetics of HRP-2 detection by the new Alere™ Ultrasensitive Malaria Ag *P.f* RDT (uRDT) after treatment in a high endemic setting has not been extensively evaluated. We performed weekly uRDT testing on children treated with 3-days or 5-days of artemether-lumefantrine (AL) and followed for 42-days in Busia, Uganda. Preliminary analysis demonstrates that 31% (13/42) of children were uRDT-negative on day 7 following treatment. Of 32 children who did not have parasitemia detectable by microscopy throughout 42-days of follow-up, only 7 (22%) remained uRDT-negative throughout the six-week period. There was no statistical difference in the duration of HRP-2 detection between AL treatment durations. We further assessed whether uRDT testing can detect HRP-2 prior to the microscopic detection of recurrent parasitemia. In 82/115 microscopy-positive samples, uRDT positivity preceded microscopic detection 7-days prior to the date of clinical or parasitological failure, and 72/110 microscopy positive samples were uRDT-positive 14-days prior (Fisher's exact test, p=0.0073 and p=0.048, respectively). Notably, for individuals with recurrent parasitemia occurring between day 14 and day 28, weekly uRDTs remained largely positive throughout the month. The final analysis will be expanded to the full data set including HIV-positive children and will be presented. In addition, ultrasensitive RT-PCR detection of parasites and strain genotyping will be performed to determine how uRDT positivity correlates with either sub-patent parasitemia, residual circulating HRP-2 antigen, or early recurrence.

1136

COMPETENCY ASSESSMENT OF MALARIA MICROSCOPISTS USING PROFICIENCY TESTING SLIDES IN TWO STATES OF NIGERIA

Wellington A. Oyibo[1], Sonachi Ezeiru[2], Diwe Ekweremadu[2], Temitope Ipinmoye[2], Genevieve Eke[3], Victor Adebayo[2], Olatayo Abikoye[2], Ezinne Onuba[2], Chukwudi Uche[3], Bala Audu[4]

[1]*ANDI Centre of Excellence for Malaria Diagnosis, College of Medicine of the University of Lagos, Lagos, Nigeria,* [2]*Catholic Relief Services (CRS) (Principal Recipient of the Global Fund, Malaria in Nigeria), Federal Capital Territory, Abuja, Nigeria,* [3]*Independent Consultant, Abuja, Nigeria,* [4]*National Malaria Control Programme, Federal Ministry of Health, Abuja, Nigeria*

Malaria microscopy is the "gold standard for malaria diagnosis and is one of the recommended tools for parasitological confirmation of malaria by the World Health Organization (WHO) and National Malaria Programmes. Before this policy recommendation, presumptive diagnosis and treatment did not permit sufficient attention on capacity strengthening efforts on malaria microscopy. We had shown in our previous studies, low to moderate performance capacity (specificity and sensitivity) for microscopy, especially the detection of low parasitaemia in peripheral blood smears, which is likely to be reported as negative. With ongoing capacity building efforts and funding of in-service training, periodic assessment of malaria microscopists in proficiency testing (PT) schemes is critical to the improvement of competency of microscopists for accurate and reliable malaria diagnosis. The competency of Malaria microscopists was assessed among 64 malaria microscopists public health facilities (HFs) in Kaduna and Delta States, Nigeria. The performance of 37 Microscopists in Kaduna state ranged from poor. Of these, two had 100% in detection, sensitivity and specificity. The lowest sensitivity, specificity and detection were 22%, 30% and 30% respectively. In Delta State, among the 27 microscopists assessed 4 scored 100% in sensitivity, specificity (100%) and detection (100%). The lowest scores for sensitivity, specificity and detection were 22%, 35%, and 35% respectively. The aggregate performance of the microscopists in Kaduna state was: sensitivity (73.6%), specificity (49.6%), detection (63%), and counting (0%); while in Delta State, sensitivity was 59.3%, specificity (60.5%), detection (59.8%), counting (0%). Appropriate training intervention and regular supportive supervision is recommended. This is critical in getting HFs prepared to handle emerging challenges such as the spread of deletions in the histidine-rich-protein -2 gene in *Plasmodium falciparum*. As interventions are scaled up, most malaria RDTs will be negative far below the limit of detection that would require high quality microscopy.

1137

ADDED VALUE OF ULTRA-SENSITIVE RDT FOR MALARIA SURVEILLANCE IN ENDEMIC AFRICA IN THE CONTEXT OF RAPIDLY CHANGING EPIDEMIOLOGY

Xiaoming Wang[1], Daibin Zhong[2], Wilfred Otambo[3], Harrysone Atieli[3], Guiyun Yan[2]

[1]*University of California, Irvine, Irvine, CA, United States,* [2]*University of California, Irvine, IRVINE, CA, United States,* [3]*Tom Mboya University, Homa Bay, Kenya*

Sensitive surveillance method is critical to the planning of malaria interventions and to the evaluation of the impact of these interventions. In the past decades many African countries have witnessed large reduction of malaria incidence, but the impact of the intervention differs greatly among countries. Consequently, the goal of malaria management varies among countries where some countries aim at reducing malaria burden, and others have shifted from malaria control to elimination. Such rapidly changing malaria epidemiology requires sensitive malaria parasite

detection tools at the point-of-care and for community-based surveillance program. This study evaluated whether this is any added value of using recently developed ultra-sensitive RDT (us-RDT) that has a lower limit of detection of *Plasmodium falciparum* parasitemia than the regular *P. falciparum* RDT. The study was carried out in Kisumu county with very high transmission and in Homa Bay county with much reduced malaria due to the indoor residual program in western Kenya. Three malaria RDTs (Alere™ us-RDT, CareStart™ PfHRP2- and PfLDH- RDTs) were examined in 400 samples from the health center settings and 800 samples from the community settings. We found that in the community setting in Kisumu county, microscopy detected 12.5% prevalence, whereas Alere™ us-RDT yielded 30.8% prevalence, significantly higher than PfLDH- RDT (17.3%) and PfHRP2 RDT (25.3%). Using Alere™ us-RDT as gold standard, PfHRP2- RDT showed 81.5% sensitivity and 100% specificity, and PfLDH- RDT exhibited 54.0% sensitivity and 99.3% specificity. In comparison to 18s rDNA qPCR, Alere™ us-RDT gave 10.4% false positive rate. In the health care setting, PfHRP2- RDT showed 84.7% sensitivity and 100% specificity, and PfLDH- RDT exhibited 71.5% sensitivity and 100% specificity, with 15.0% false positive rate in comparison to 18s rDNA PCR. These results suggest us-RDT provided some added value in detection malaria infection in the community. We are currently analyzing the samples from Homa Bay where transmission was reduced by several folds in the past two years to assess the utility us-RDT in low transmission settings.

1138

PLASMODIUM FALCIPARUM INFECTION IN TRANSFUSION MEDICINE: EVALUATION OF SAFE BLOOD DONATION

Abimbola Amoo

University College Hospital, Ibadan, Nigeria

In sub-Saharan Africa, Malaria could lead to about 770, 000 death alone this year if COVID-19 disrupts the key strategies, according to a newly available report on WHO modeling analysis. Transfusion medicine infections are mostly prevalent studies on bacteria and viruses with very little or no effort at investigating malaria parasite infection that can cause severe post-transfusion illness, especially in transfusion-dependent patients. This study designed to bridge the gap by screening for *Plasmodium falciparum* infection and determine the effect of storage duration at 4°C on *Plasmodium falciparum* infection. A cross-sectional study on blood donors in Ibadan Oyo state, National blood transfusion service centre. Demographic data and clinical history were obtained using a pro-formal questionnaire. Donor's blood samples were *P. falciparum* positive using Giemsa stained microscopy. A total of 248 blood samples collected from donors from southwest Nigeria were tested for malaria parasites. The overall prevalence of *P. falciparum* infection using Giemsa microscopy was 8.5%. The prevalence in blood samples stored for 3days, 7 days, and 21 days were 8.1%, 7.3%, and 5.7% respectively. There was a significant decline in the prevalence of *P. falciparum*, by microscopy, with an increased period of blood storage from day 0 to day 21 (Kendall's Tau; $p < 0.001$). The present study revealed that *P. falciparum* is prevalent among blood donors in the Ibadan, Nigeria. Blood storage for about 21 days can significantly reduce the risk of transfusion-transmitted *P. falciparum* malaria. This can be attributed to the lack of adequate accommodation and poor sanitary conditions in the area under study. National surveillance and public health education should stop the spread of parasitic infections in transfusion medicine.

1139

RISK FACTORS FOR MALARIA IN HIGH INCIDENCE AREAS OF VIET NAM: A CASE-CONTROL STUDY

Richard J. Maude[1], Ipsita Sinha[1], Kulchada Pongsoipetch[1], Rapeephan R. Maude[1], Dang Viet Dung[2], Nguyen Huong Binh[2], Tran Thanh Duong[2], Ngo Duoc Thang[2], Nicholas J. Martin[3]

[1]*Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand,* [2]*National Institute of Malariology, Parasitology and Entomology (NIMPE), Hanoi, Viet Nam,* [3]*Naval Medical Research Unit-2, Singapore, Singapore*

Viet Nam aims to eliminate malaria by 2030. Key to achieving this goal is having a detailed understanding of which groups of people are most at risk to guide efficient targeting of limited resources towards treatment and prevention. One thousand unselected malaria positive cases and age, gender-matched malaria negative controls were recruited at commune health stations (CHS) in Binh Phuoc, Dak Nong and Dak Lak Provinces. Diagnosis was confirmed with microscopy, rapid diagnostic test and PCR. Participants were interviewed about 50 potential risk factors for malaria including occupation, visiting the forest, travel, healthcare seeking behavior and prior use of interventions. Participants were enrolled by trained government health workers and the samples analysed in the government laboratories in Viet Nam. Data were analysed by univariable, block-wise and multivariable logistic regression. Among cases, 61.8% had *P. falciparum*, 35.2% *P. vivax* and 3% mixed species. Median (IQR) age was 27 (21-36) years and 91.2% were male. Twenty five risk factors were associated with being a case and 11 with being a control. In a multivariable logistic regression, being a forest worker, having visited the forest in the previous 2 months, longer duration of illness, having a recorded fever, number of times they had malaria in the past year, having had prior malaria treatment and having previously visited a government or private sector clinic were associated with being a case. This study demonstrates the increased statistical power from matching cases and control by age and gender where these are known to be strongly associated with malaria risk. The increased statistical power from this approach allowed identification of additional independent risk factors. It also illustrates an example of research partnership between academia and government to collect high quality data relevant to planning malaria elimination activities. Modifiable risk factors and implications of the findings for malaria elimination strategy are discussed.

1140

LONGITUDINAL ASSESSMENT OF THE HUMAN INFECTIOUS RESERVOIR IN AN AREA UNDER INTENSIVE MALARIA CONTROL: PERSISTING INFECTIOUSNESS TO MOSQUITOES AND SUPER SPREADERS

Chiara Andolina[1], John Rek[2], Jessica Briggs[3], Joseph Okoth[2], Alex Musiime[2], Emmanuel Arinaitwe[2], Melissa Conrad[3], Joaniter Nankabirwa[2], Kjerstin Lanke[1], Lisette Meerstein-Kessel[1], Peter Olwoch[4], Moses Kamya[2], Grant Dorsey[3], Bryan Greenhouse[3], Chris Drakeley[5], Sarah G. Staedke[6], Teun Bousema[1]

[1]*Radboudumc, Nijmegen, Netherlands,* [2]*The Infectious Diseases Research Collaboration (IDRC), Kampala, Uganda,* [3]*Department of Medicine, University of California San Francisco, San Francisco, CA, United States,* [4]*The Infectious Diseases Research Collaboration (IDRC) i, Kampala, Uganda,* [5]*Department of Immunology and Infection, London School of Hygiene & Tropical Medicine, London, United Kingdom,* [6]*Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London, United Kingdom*

Our knowledge on how malaria is transmitted to mosquitoes is still incomplete. Identifying the reservoir of malaria infection is crucial to develop and improve interventions aimed to reduce the burden of malaria to its ultimate elimination. To longitudinally quantify the contribution of asymptomatic and symptomatic individuals to transmission in an area under effective malaria control, we performed an all-age cohort study in 531 individuals over 24 months in Tororo district, eastern Uganda. *Plasmodium falciparum* parasite prevalence, density and genotypes were

determined every four weeks by ultrasensitive varATS qPCR and targeted deep sequencing. A total of 572 membrane feeding experiments were conducted: 24 on clinical malaria cases and 413 during asymptomatic infections. Mosquito infections were observed in 6.8% (39/572) experiments with 1.12% (446/39,801) of mosquitoes infected and an infection range of 1.06% to 81.39 %. Asymptomatic children aged 5-15 years comprised the majority of infectious individuals (82,05%, 32/39). Total parasite density was positively associated with mosquito infection rates among asymptomatic parasite carriers (ρ=0.539, P<0.001) but not among clinical malaria cases (ρ=0.0145, P=0.93). Only 35.9% of infectious donors harboured parasite densities >100p/uL, the routine microscopy detection threshold. During the study period, parasite prevalence, density and clonal complexity declined. In individuals with repeated membrane feeding assays, this decline in parasite burden was mirrored by declining likelihood of infecting mosquitoes. Two individuals remained infectious for more than six months of follow-up. One individual who was infected with seven *P. falciparum* genotypes and infectious on eight occasions, was responsible for 24.7% of all infected mosquitoes observed among the cohort. In this unique longitudinal study, we find that asymptomatic infections in school aged children are responsible for the majority of onward transmission events, infectious gametocytes persist for several months among asymptomatic infections and a minority of individuals is highly infectious to mosquitoes.

1142

PLASMODIUM FALCIPARUM PREVALENCE, SPATIAL DISTRIBUTION, AND SOCIO-DEMOGRAPHIC FACTORS ASSOCIATED WITH INFECTION IN SUSSUNDENGA, MOZAMBIQUE ALONG THE ZIMBABWE BORDER

João L. Ferrão[1], Dominique Earland[2], Anísio Novela[3], Roberto Mendes[4], Marcos F. Ballat[5], Alberto Tungadza[4], Albino Bibe[6], Valy Muhiro[7], Kelly M. Searle[2]

[1]*Instituto Superior de Ciências de Educação, Beira, Mozambique,* [2]*University of Minnesota, Minneapolis, MN, United States,* [3]*Direcção Distrital de Saúde de Sussundenga, Manica, Sussundenga, Mozambique,* [4]*Universidade Católica de Moçambique em Beira, Beira, Mozambique,* [5]*Universidade Católica de Moçambique em Chimoio, Chimoio, Mozambique,* [6]*Escola Secondária de Sussundenga, Sussundenga, Mozambique,* [7]*Departamento de Malaia da Direção de Saúde de Sussundenga, Sussundenga, Mozambique*

Malaria is one of the leading causes of morbidity and mortality in Mozambique with the 5th highest prevalence in the world and limited progress in control over the past 20 years. Sussundenga Rural Municipality in Manica Province is one of most impacted areas, and lies along the Zimbabwe border, making evaluation of transmission and control policies integral for regional efforts. The objective of this study was to quantify *Plasmodium falciparum* parasite prevalence, map the spatial distribution of infections, and model its relationship with socio-demographic factors in Sussundenga Rural Municipality. Homes in the study area were digitalized and enumerated using GoogleEarth Pro$_{TM.}$ A sample of 125 houses was drawn to conduct a community survey of *P. falciparum* prevalence using a rapid diagnostic test (RDT). A map *P. falciparum* prevalence of was produced. A questionnaire was administered to assess the socio-demographic characteristics of participants. Logistic regression was performed to establish the relationship between infections and the socio-demographic characteristics (sex, age, employment, education, population density, household size, location, previous history of malaria treatment, and cell phone ownership). Three-hundred, forty-two participants completed the survey. The overall *P. falciparum* prevalence was 32.5 %. Mapping showed evidence of distinct areas of high, moderate, and low malaria prevalence. *P. falciparum* prevalence was highest in the 5-14 year-old age group (50.5%), which was significantly higher than other age groups (p<0.001). The model determined that education level, population density, age category, and location were associated with *P. falciparum* infection in this population. There was a 7.8- fold increase in the odds of infection for participants in larger households compared to smaller households. The model explained 31.4% *of the variance and correctly classified 76.1 % of infections.* In this area, *P. falciparum* infection was highest among school-aged children and highly related to socio-demographic factors and location. These finding can be used to guide more effective interventions in this region.

1143

DYNAMICS OF SUBCLINICAL MALARIA DETECTED BY ULTRASENSITIVE PCR IN DIFFERENT TRANSMISSION ZONES OF MYANMAR AND ALONG ITS BORDER WITH CHINA

Joseph Egger[1], Kay T. Han[2], Huang Fang[3], Xiao-Nong Zhou[3], Tin M. Hlaing[4], Myo Thant[4], Xiao X. Wang[3], Tu Hong[3], Ryan Simmons[1], Thynn K. Thane[1], Manfred Meng[1], Joyce Hogue[1], Christine F. Markwalter[1], Aung Thi[5], Thura Htay[1], Zaw W. Thein[1], Zin M. Tun[1], Swai M. Oo[1], Alyssa Platt[1], Poe P. Aung[1], Christopher V. Plowe[1], Myaing M. Nyunt[1]

[1]*Duke Global Health Institute, Duke University, Durham, NC, United States,* [2]*Department of Medical Research, Myanmar Ministry of Health and Sports, Yangon, Myanmar,* [3]*National Institute of Parasitic Diseases, China CDC, Shanghai, China,* [4]*Defense Services Medical Research Center, Myanmar Ministry of Defense, Naypyitaw, Myanmar,* [5]*National Malaria Control Program, Myanmar Ministry of Health and Sports, Naypyitaw, Myanmar*

The recent recognition of a large asymptomatic malaria reservoir elimination in the Greater Mekong Sub-Region has raised new questions about malaria in this low transmission setting. The vast majority of these infections are missed by standard malaria diagnostics and can be detected only by ultrasensitive PCR (usPCR) which is thousands-fold higher more sensitive. Very little is known about these low-density subclinical malaria infections, including whether they are transient or persist over time. A prospective longitudinal cohort study was conducted to evaluate the dynamics of usPCR-positive infections in three transmission zones: 1) Rakhine State (high transmission, *P. falciparum* and *P. vivax* co-dominant), 2) Sagaing Region (low transmission, unknown species dominant) in Myanmar, and 3) Nabang Township, on the Myanmar-China border (low transmission, *P. vivax* dominant). Biweekly or weekly testing was done six or two times for participants who were RDT-negative and usPCR-positive, or RDT-negative and usPCR-negative at enrollment, respectively. Overall, 96%, 87% and 87% of 2,709 enrolled participants completed the study, and 9.6%, 2.3% and 9.6% were usPCR-positive at enrollment at sites 1, 2 and 3, respectively. In site 1, 37.8% and 32.8% of *P. falciparum* and *P. vivax* positive participants remained positive in all six consecutive observations. In sites 2 and 3, *P. vivax* was the major species, and 14.3% and 19.8% of usPCR-positive at enrollment remained positive for all six observations in site 2 and 3. At least 49% of participants who were usPCR-positive at enrollment remained positive for at least three of six observations. More than 95% of usPCR-negative participants remained negative for the second observation. The major factors associated with malaria positivity at enrollment included older age, longer distance between work and home, infrequent bednet use, more frequent travel outside of home, or trips to the forest or water source. The study data were used to design an ongoing longitudinal matched cohort study to assess clinical and transmission risks of subclinical low-density malaria in different epidemiological settings.

1144

IMPORTATION OF HAPLOTYPES MAY DRIVE MALARIA EPIDEMICS IN A HIGHLAND AREA OF WESTERN KENYA WITH UNSTABLE TRANSMISSION

Lindsey B. Turnbull[1], Kelsey M. Sumner[2], Hannah Meredith[3], Elizabeth Freedman[4], George Ayodo[5], Joseph Saelens[4], Steve M. Taylor[4], Chandy C. John[1]

[1]*Ryan White Center for Pediatric Infectious Diseases and Global Health, Indianapolis, IN, United States,* [2]*Gillings School of Public Health, Chapel*

Hill, NC, United States, [3]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [4]Duke University School of Medicine, Chapel Hill, NC, United States, [5]Kenya Medical Research Institute, Kisumu, Kenya

Sustainable elimination of malaria in areas with very low incidence requires identifying remaining sources of infection and targeting interventions to these sources. Due to waning immunity, it is important to identify new, imported parasite genotypes as they may pose an increased risk to susceptible populations, resulting in an outbreak. In our study area in the Nandi Highlands of Western Kenya, annual clinical malaria incidence was at pre-elimination levels (<10 cases/1000 residents) until an epidemic in 2017, in which cases increased more than 4-fold. As both clinical incidence and asymptomatic parasitemia prevalence were very low prior to the 2017 epidemic, we hypothesized that the epidemic was due in part to importation of new malaria genotypes. However, no study participant with clinical malaria during this time period reported overnight travel outside of the study area in the 30 days prior to visiting the health facility. To further investigate the possibility of importation of malaria parasites, we assessed differences in parasite genotype over time. We deep sequenced two polymorphic *P. falciparum* amplicons, AMA-1 and CSP, in all qPCR positive clinical malaria patients who visited the health facilities from 2012 - 2017 (n=372). Using the sequenced reads, we designated haplotypes for AMA-1 and CSP that represented genetically distinct malaria infections. The median multiplicity of infection by both AMA-1 and CSP was 2.0 and did not differ across years. In the epidemic year (2017), the prevalence of new haplotypes, i.e., haplotypes not seen in the previous year, increased significantly from 12.0 and 41.7% for CSP and AMA in 2016 to 66.9 and 72.6% in 2017 (χ^2, CSP p <0.00001, AMA-1 p=0.0059). A significant difference in the proportion of new haplotypes was also observed during a peak in clinical malaria in 2013 (61.9% CSP, 72.7% AMA) compared to years without peaks (mean= 30.0% CSP, 41.8% AMA) (χ^2, CSP p=0.025, AMA-1 p=0.0023). The study findings suggest that the presence of parasite genotypes new to the study population, imported by vector and/or human travel, may have contributed to increases in incidence, including the 2017 epidemic.

1145

MALARIA TREATMENT-SEEKING BEHAVIOR FROM MALARIA AT COYAH DISTRICT HOSPITAL, GUINEA

Alioune Camara[1], Timothee Guilavogui[2], Mamadou Oury Cissé[1], Aboubacar Sidiki Conde[3], Aissata Fofana[4], Donatien Ntambue[5], Eugene kaman Lama[1], Alexandre Delamou[3]

[1]*National Malaria Control Program, Conakry, Guinea, [2]Direction of Major endemics and Disease Control, Ministry of Health, Conakry, Guinea, [3]Department of Public Health, Gamal University of Conakry, Conakry, Guinea, [4]RTI International, Conakry, Guinea, [5]Catholic Relief Services (CRS), Conakry, Guinea*

Currently less than 18% of children with fever and malaria receive in Guinea recommended artemisinin-based combination therapy (ACT). To understand why coverage remains low, it is necessary to examine the care-seeking pathway from start of febrile illness to receiving appropriate treatment. A cross-sectional study was conducted among 600 malaria patients of all ages seeking treatment from February to June 2019 in Coyah District Hospital in Guinea. Socio-demographic characteristics and the reported treatment-seeking pathway were described. Treatment-seeking sources were categorized as conventional (health centre, private establishment, hospital) or alternative (self-medication, traditional/herbal). Logistic regression was used to determine the predictors of delay in seeking care at health facilities (>48 hours after onset of fever). Most patients surveyed were over 5 years old (63.8%), male (56.3%) and residing in rural areas (77.3%). When considering the first recourse for healthcare in the interviewed malaria patients, 39% of the patients had sought conventional treatment, 17% had sought traditional/herbal treatment and 44% had self-medicated. A total of 75.5% of patients had received malaria treatment by their third treatment-seeking visit. The most common treatment-seeking pathways included use of hospital alone (10.7%), private institutions then hospital (12.2%), health centres then hospital (9.5%), and self-medication then private institutions and hospital (9.2%). Use of alternative sources was reported by 63.2% of the patients. Only 143 (23.8%) of participants sought treatment in health facilities within 48 hours of the onset of fever. Diagnosis of severe malaria [adjusted OR (ORa) = 2.56, 95% Confidence Interval: 1.41 - 4.64], malaria associated with another disease [ORa = 2.43: 1.34 - 4.42] and alternative first response [ORa = 2.89: 1.95 - 4.29] were associated with delayed use of health facilities. Conclusions: More accessible and affordable health services and improved behavior change communication programs are needed to increase the demand for formal providers.

1146

PLASMODIUM FALCIPARUM GAMETOCYTE SEX RATIOS IN CHILDREN AND ADULTS WITH ASYMPTOMATIC, LOW-DENSITY INFECTION IN TANZANIA

Meredith Muller

University of NC Chapel Hill, Chapel Hill, NC, United States

Both male and female gametocytes must be ingested in a mosquito blood meal for successful *Plasmodium falciparum* transmission from humans to mosquitos. Since male gametocytes give rise to multiple gametes, it is logical that gametocyte sex ratios are generally female-biased. At low densities, this ratio may need to be more balanced to ensure transmission to mosquito hosts. We describe sex-specific gametocytemia in a cohort of asymptomatic children and adults in Bagamoyo, Tanzania, of which 60% (127/210) were smear-negative and 34% were RDT-negative. In this cohort, adults were more likely to have low-density infections with 81% of adults (>18yo) vs 55% of children (<18yo) harboring parasite densities <100 parasites/uL, as measured by 18s qPCR. RNA extracted from capillary blood was screened by quantitative reverse transcriptase PCR assays targeting gametocyte sex-specific markers containing introns:PF3D7_0630000 (female target, limit of detection (LOD)=1 gametocyte/uL) and PfMGET (male target, LOD=0.1 gametocytes/uL). Even though only 5% (11/210) of subjects had patent gametocytes, 68% (143/210) had gametocytes detectable by RT-qPCR. When examining only infections with <100 parasites/ul, gametocytes were detected in 55% (75/136) of subjects. Of the gametocytemic subjects, 54% (77/143) had both sexes of gametocytes, while in all but one (45% or 65/143), only male gametocytes were detected, likely owing to the lower LOD of the male assay. Among those with both sexes of gametocytes detected (n=77), the density of each sex was found to be positively correlated (r=0.99, p<0.0001). After removing samples with <1 male gametocyte/uL (n=57), as hypothesized, the percentage of male gametocytes was higher at lower total gametocyte densities (ANOVA, p=0.001). This was also the case at lower parasite densities (ANOVA, p=0.01). There was no significant difference in gametocyte sex ratios for male and female participants, but younger participants were more likely to have female-biased gametocyte sex ratios (p=0.001). Our findings shed light on characteristics of the asymptomatic infectious reservoir in an area of low to moderate malaria transmission.

1147

MALARIA PREVALENCE AND GAMETOCYTE CARRIAGE IN ENDEMIC AREA OF BANCOUMANA, MALI, A MALARIA VACCINE TESTING SITE

Sibiri Sissoko[1], Jen C.C. Hume[2], Mahamadoun H.A. Maiga[1], Kourane Sissoko[1], Seydou Sankare[1], Sadio K. Diarra[1], Boukary Togo[1], Abdoulaye Djiguiba[1], Amatigue Zeguime[1], Sintry Sanogo[1], Moussa B. Kanoute[1], Aissata D. Doumdia[1], Bourama Samake[1], Drissa Dembele[1], Aly Togora[1], Adama Outtara[1], Amadou D. Barracko[1], Korotoumou Goita[1], Alemush Imeru[3], Patrick E. Duffy[3], Issaka Sagara[1]

[1]*Malaria Research and Training Center, University of Science, Techniques and Technologies, Bamako, Mali, [2]Laboratory of Malaria Immunology*

and Vaccinology, Bethesda, MD, United States, [3]Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States

Demographic characteristics, geographic location and seasonal variation can influence the risk of malaria infection. In Bancoumana, Mali (where malaria transmission is seasonal from July to Dec), we implemented a cohort study from Feb 2018 to Jan 2020 to characterize transmission dynamics and assess malaria infection and gametocyte carriage rates. 830 participants aged 6 months to 65 years were recruited and completed scheduled monthly visits for clinical and laboratory assessments, and 20,936 blood smears were collected. The frequency of malaria infection was 14.8% (1580/10701) vs 12.8% (1309/10235) in successive years. Positive blood smears in Year 1 and Year 2 were most frequent for *Plasmodium falciparum* (94.2% and 93.4%), followed by *Plasmodium malariae* (2.3% and 2.2%), mixed infections (2.7% and 4.4%), and *P. ovale* (0.75% and 0.03%), respectively. Only *P. falciparum* gametocytes were recorded. Gametocyte carriage rate was 3.1% (332/10701) in Year 1 and 1.6% (161/10235) in Year 2 (p<0.001). Gametocytemia was more frequent in males (4.0% (206/5108) in Year 1 and 2.1% (102/4828) in Year 2) than females (2.25% (126/5593) in Year 1 and 1.09% (59/5407) in Year 2) (P <0.001). Peak gametocytemia rates varied: 7.7% (67/867) in Feb 2018 with another peak of 4.4% (41/938) in Nov 2018; then 5.01% (41/818) in Dec 2019. Despite malaria seasonality, gametocyte carriage rates were similar between wet and dry seasons in Year 1 (Wet: 3.20% Vs Dry: 3.01%; P=0.577) but differed in Year 2 (Wet: 1.90% Vs 1.12%; P=0.002). Year 1 and Year 2 gametocyte carriage rates by age groups were (respectively): 1.3% and 1.09% (<5 years), 4.0% and 1.5% (5-10 years), 4.4% and 2.4% (11-17 years), and 2.1% and 1.0% (≥18 years). *P. falciparum* infection rate was highest in children aged 11-17 years. The low malaria infection and gametocyte carriage rates in children <5 years was likely due in part to seasonal malaria chemoprevention and bednet use. The persistence of *P. falciparum* gametocytes throughout the year enables rapid malaria transmission when mosquito populations increase in the rainy season.

1148

COMPARING SEASONAL DYNAMICS OF MALARIA EPIDEMIC IN CAMEROON TO INFORM MALARIA PRE-ELIMINATION EFFORTS

Akindeh Mbuh Nji[1], Innocent M. Ali[1], Peter Thelma Ngwa[1], Jude Diaga Bigoga[1], Sheetal Silal[2], Wilfred Fon Mbacham[1]

[1]*MARCAD Program -University of Yaounde I, Yaounde, Cameroon, Cameroon,* [2]*Faculty of Statistical Science, University of Cape Town, Cape Town, South Africa*

Plasmodium falciparum malaria is a public health problem in tropical countries with 247 million cases annually occurring globally and 86% in Africa. It is known as a major public health problem in Nigeria where it accounts for more cases and deaths than any other country in Africa. Malaria is a risk for about 97% of Cameroon's population. However, the seasonal dynamics of this disease from place to place are poorly characterized and many questions remain unanswered about the ecology of the disease. In this paper we assess comprehensively the seasonal trends in Malaria incidence in Cameroon, estimated and compared spatial synchrony and lag time between epidemics in different locations over time. Monthly incidence data was collected from Cameroon's National Malaria Control Program. We performed wavelet analysis to identify whether annual seasonality is detected in time series that had at least 4 years of data with more than 50,000 cases annually. We estimated the synchrony between epidemics in different locations and the coherency between two sets of signals over time. A persistent seasonality was observed in different regions through the wavelet analysis approach. The regions shared different seasonality and time-lag differences especially between each other. It was confirmed that there are cycles lag and higher synchrony of annual malaria cycles in nearby states. The northern regions of Cameroon lag behind those of the South. The results provide significant insight into the seasonal dynamics of malaria epidemiology in Cameroon. Understanding these seasonal patterns is very crucial in deciding the different periods to roll out control efforts and the best intervention combinations for malaria pre-elimination strategies in Cameroon.

1149

SYNERGY BETWEEN MALARIA MAPS AND GENOMIC DATA COLLECTION IN SENEGAL: REFINING OUR UNDERSTANDING OF TRANSMISSION

Katherine E. Battle[1], Albert Lee[1], Jonathan Russell[1], Jessica Ribado[1], Ewan Cameron[2], Punam Amratia[3], Justin Millar[3], Tasmin Symons[3], Rohan Arambepola[3], Fatou Ba[4], Medoune Ndiop[4], Alioune B. Gueye[4], Ibrahima Diallo[4], Doudou Sene[4], Daouda Ndiaye[5], Bronwyn MacInnis[6], Dyann Wirth[6], Sarah Volkman[6], Peter W. Gething[2], Edward Wenger[1], Joshua L. Proctor[1], Caitlin Bever[1]

[1]*Institute for Disease Modeling, Bellevue, WA, United States,* [2]*Telethon Kids Institute, Perth, Australia,* [3]*University of Oxford, Oxford, United Kingdom,* [4]*Senegal National Malaria Control Program (PNLP), Dakar, Senegal,* [5]*Cheikh Anta Diop University, Dakar, Senegal,* [6]*Broad Institute of Harvard and MIT, Cambridge, MA, United States*

Senegal has the opportunity to demonstrate how malaria parasite genomic data can be incorporated into practical country level decision making. As a multi-year collaborative study gets underway between the national malaria control program and its research and academic partners, there is an opportunity to leverage Senegal's robust routine health system and rolling Demographic Health Survey data to inform the selection of sites where genomic data will be gathered. Maps of prevalence and incidence have demonstrably informed planning and sampling efforts for malaria in the past. Local high-resolution data from Senegal indicate that malaria transmission can vary considerably even across small geographical regions and temporal windows. Variation in transmission is demonstrated in the arrondissement of Makacolibantang (Tambacounda District) where cases observed at weekly cadence at the health facility level can be linked to surrounding villages. Bespoke fine scale maps modelled using both prevalence and incidence data can inform understanding of how transmission varies and where sampling will ideally occur in order to illustrate the relationship between genetic features and malaria metrics. The link between genetic features and transmission metrics has been demonstrated from initial barcode data from existing sentinel sites. Preliminary mapping indicates coarse heterogeneity across the county, but also fine scale variation within arrondissements. As data collection progresses, there will be a unique opportunity for the examined genetic features to refine maps of prevalence and incidence and improve understanding of how parasites may be moving within the country. Patterns of movement will help reveal regions where transmission is maintained by importation. Outputs of this nature will be particularly useful to Senegal, where ambitions for pre-elimination certification are on the horizon.

1150

LOW INCIDENCE OF CLINICAL MALARIA IN UNDER-FIVES IN BANCOUMANA, MALI, A MALARIA VACCINE TESTING SITE

Kourane Sissoko[1], Jen C.C. Hume[2], Mahamadoun H. Assadou[1], Amatigue Zeguime[1], Sibiri Sissoko[1], Mamady Kone[1], Seydou Sankare[1], Sadio K. Diarra[1], Boukary Togo[1], Abdoulaye Djiguiba[1], Sintry Sanogo[1], Moussa B. Kanoute[1], Aissata D. Doumdia[1], Bourama Samake[1], M'Bouye Doucoure[1], Drissa Dembele[1], Aly Togora[1], Adama Ouattara[1], Modibo Traore[1], Alemush Imeru[2], Patrick E. Duffy[2], Issaka Sagara[1]

[1]*Malaria Research and Training Center, Bamako, Mali,* [2]*Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States*

Effective surveillance of malaria cases and deaths is essential for identifying the areas or population groups that are most affected by malaria, and for targeting resources for maximum impact. An effective malaria vaccine remains an urgent priority to reinforce the current tools for elimination.

The present analysis was undertaken to determine the clinical malaria incidence in a general population living in a malaria endemic area, to inform future evaluations of malaria vaccine candidates. We conducted a cohort study in a malaria endemic area, Bancoumana, Mali, a malaria vaccine testing site. A total of 830 volunteers aged 6 months to 65 years old were enrolled in this study of community dynamics of malaria transmission. Study participants were seen monthly and at the time of any illness by study clinicians. Malaria blood smears and/or rapid diagnostic tests (RDTs) were performed upon observation of clinical symptoms to confirm clinical malaria before treatment initiation. Malaria incidence data collected from February 2018 to January 2020 are presented here. For 2018 the incidence rate of clinical malaria across all ages was 0.63 per person-year (505 cases/801.9 person-year): 0.34 (46/134.8) for under-fives, 0.74 (162/219.1) for 5-10 years, 0.88 (174/198.3) for 11-17 years and 0.49 (123/249.8) for ≥18 years. In 2019 the incidence rate of clinical malaria across all ages was 0.76 (573/753.5) per person-year: 0.53 (52/97.3) for under-fives, 0.89 (204/229.3) for 5-10 years, 0.99 (207/209.5) for 11-17 years and 0.51 (110/217.3) for ≥18 years. The incidence of malaria is highly seasonal and continues to be high in the general population in the study area, with the greatest burdens in children aged 5-17 years. The disproportionately low incidence in under-fives is likely explained by seasonal malaria chemoprevention distributed during the high malaria transmission season (August to November). The incidence of malaria was higher in 2019 than 2018 (p=0.015). These seasonal and age patterns of the malaria burden should be considered when evaluating new interventions such as vaccines or other new control strategies.

1151

SPATIAL HETEROGENEITIES IN MALARIA TRANSMISSION INTENSITY, INSECTICIDE TREATED NET USE AND ACCESS IN NIGERIA AND ASSOCIATED FACTORS

Ifeoma D. Ozodiegwu, Jaline Gerardin

Northwestern University, Chicago, IL, United States

Nigeria is one of the countries with the highest malaria burden globally, accounting for roughly 24% and 25% of global malaria deaths and cases respectively in 2018. Nigeria currently has operational plans for targeted deployment of vector control tools but the question of where to consider withdrawing insecticide treated nets (ITN), given the highly heterogeneous nature of malaria transmission, remains unanswered. Presently, data to inform such decisions at finer resolutions is lacking. In particular, literature gaps exist in adequate comprehension of the urban/rural variability in malaria transmission intensity, insecticide treated net use and access in the same geographic region, and their drivers. Moreover, whether the drivers of ITN access and use varies at the same level of malaria transmission intensity is not well understood. As such, we aimed to examine spatial heterogeneities in transmission intensity and ITN access and use within urban and rural strata of Nigeria's six geopolitical zones and identify correlated factors. A secondary objective of our work was to identify the determinants of ITN use and access at the same levels of transmission intensity. We conducted our analysis using data from the 2018 Demographic and Health Survey, enhancing the relevance of findings to current policy decisions in Nigeria. Our preliminary findings indicate higher average levels of *Plasmodium falciparum* parasite rates (*Pf*PR), among children aged 0 – 5 years, in rural areas compared to the urban areas, except in the South East, where results were similar. Negative correlations were observed between the proportion of individuals in rich wealth quintiles and cluster-level *Pf*PR in both urban and rural areas, with the South East urban areas having the greatest correlation coefficient (r = -0.47; - 0.40, - 0.53). The findings of our study could potentially support future programmatic decisions on the optimal allocation of ITN by providing insight into areas where they will have minimal impact while informing programming to maximize their impact in areas of greatest need.

1152

CO-INFECTION OF *PLASMODIUM FALCIPARUM* AND HELMINTHS AMONG SCHOOL CHILDREN IN COMMUNITIES IN SOUTHERN AND NORTHERN GHANA

Gabriel Akosah-Brempong, Simon K. Attah, Yaw A. Afrane

University of Ghana Medical School, Accra, Ghana

Malaria, Schistosomiasis and Soil transmitted helminth (STH) infections inflicts significant burden on children mostly in deprived communities in Ghana. Despite the deployment of malaria vector control and annual Mass Drug Administration by National Control Programmes, these infections still pose major public health concerns in Ghana. Adequate data is necessary for policy formulations and strengthening of interventions to mitigate transmission. This study assessed the co-infection burden of *Plasmodium, Schistosoma* species and STH infections among school children in communities in Southern and Northern Ghana. School children living in communities in Southern (Anyarkpor, Pediatorkope, Tuanikope) and Northern Ghana (Kpalsogu) were enrolled in a cross-sectional study. Stool samples were collected to screen for *Schistosoma mansoni* and STH infections using formol-ether concentration technique and urine samples were examined for *S. haematobium* using the centrifugation method. *Plasmodium* parasitaemia was determined from thick and thin finger prick blood samples. Out of the 493 school children enrolled in the study, overall prevalence of *P. falciparum, S. mansoni, S. haematobium, Trichuris trichiura* and hookworm infections were 17.2%, 22.6%, 1.6%, 1.2% and 1.2% respectively. *Plasmodium falciparum* infection was widespread in all the study sites with higher burden in the Southern communities (20.9%) than the Northern community (5.9%). *Schistosoma. mansoni* was highly present only in the southern communities (27.4%). A total of 4.5% children were co-infected with these parasites, occurring only in the Southern communities; of which combination of *P. falciparum* and *S. mansoni* was predominant (1.4%). We identified a relatively low burden of parasites co-infection among the children studied, however, there were high prevalence of single infections of *P. falciparum* and *S. mansoni* in the Southern communities

1153

LONG-TERM PREDICTION OF MALARIA IN MOZAMBIQUE USING INTERANNUAL CLIMATE VARIABILITY

James Colborn[1], Ryan Harp[2], Kathryn Colborn[3], Baltazar Candrinho[4], Kris Karnauskas[2]

[1]*Clinton Health Access Initiative, Maputo, Mozambique,* [2]*University of Colorado Boulder, Boulder, CO, United States,* [3]*University of Colorado Anschutz Medical Campus, Aurora, CO, United States,* [4]*Mozambique National Malaria Control Program, Maputo, Mozambique*

Malaria is among the biggest public health problems in Mozambique, with over 10 million cases reported annually since 2018. Although the relationship between climate and seasonal trends in malaria cases has been well established, the role of climate in deviations from these annual patterns is less clear. To investigate this and the potential for using inter-annual climate variability to predict malaria cases, weekly district-level malaria rates spanning 2010-2017 were quality controlled and prepared for cross-analysis with climate data. The strong seasonal cycle of malaria rates was removed using a low-pass filter to expose and facilitate comparison with interannual climate variability. Principal component analysis revealed two dominant spatial patterns that account for over 80% of the interannual variability: one hot spot located over the southern half of Mozambique (64%), and another in the northern third of the country (17%). Linear regression of satellite-derived precipitation over these hotspots onto global observations of ocean temperatures links the dominant mode of interannual malarial variability to the El Niño-Southern Oscillation (ENSO), where La Niña (El Niño) leads to wetter (drier) conditions over southern Mozambique and higher (lower) disease prevalence. The time series associated with the second leading mode is strongly anti-correlated (-0.80) with the Indian Ocean Dipole (IOD) . Similar analysis of spatial patterns of precipitation over a longer time period

(1979-2019) confirms the importance of the ENSO to Mozambique rainfall but suggests that the *Subtropical* Indian Ocean Dipole (S-IOD) is actually a stronger factor for precipitation than the traditional IOD. These results illustrate the potential for a malaria early warning system that leverages quasi-predictable modes of interannual climate variability in the tropical oceans.

1154

EFFECTS OF CLIMATE CHANGE AND URBANIZATION ON MALARIA TRANSMISSION IN URBAN ETHIOPIA—A SCOPING REVIEW

Yue Yuan

University of British Columbia, Vancouver, BC, Canada

In 2017, approximately 75% of Ethiopia and 68% of its population is at risk of malaria infection. Malaria transmission depends on climate variability because both vectors and parasites are sensitive to temperature and precipitation. The climate in Ethiopia has been generally getting warmer over the last 30 years. Rising surface temperature means that the optimal temperature for transmission gradually moves up to higher altitudes, which leads to geographical expansion/shifts of the disease. In addition, as one of the fastest urbanizing nations in the world, Ethiopia experiences profound demographic, ecological, and socioeconomic changes with a high degree of spatial and temporal heterogeneity, which may significantly affect parasite-vector-human interactions in urban areas. This scoping review aims to explore how risk factors associated with climate change and rapid/unplanned urbanization affect malaria transmission in urban Ethiopia. The author searched peer-reviewed articles published in English between January 2003 and December 2019 in Ovid and Web of Science databases. Two sets of search terms were used, with the first set focusing on climate and environmental changes, and the second on urbanization. 287 articles were yielded from the search terms. After exclusion, 23 articles were included in this review. Malaria appeared to already have been detected in the highlands, where it previously was not present. Minimum monthly temperature, rainfalls and relative humidity, and extreme weathers such as draught and floods showed great statistical significance in affecting malaria transmission. Additionally, risk factors associated with rapid/unplanned urbanization such as lack of toilet facilities, insufficient housing conditions, inadequate drainage and waste disposal, and limited access to clean drinking water were reported. Proximity to stagnant water and irrigation systems, vegetation, changes in land use, deforestation, healthcare access, and socioeconomic disparities require further investigation in local contexts.

1155

SEASONAL MALARIA CHEMOPREVENTION WITH SULFADOXINE-PYRIMETHAMINE PLUS AMODIAQUINE AND GAMETOCYTE CARRIAGE IN CHILDREN WITH ASYMPTOMATIC *PLASMODIUM FALCIPARUM* INFECTIONS

Abdullahi Ahmad, Mamadou Ndiath, Aurelia Prom, Blessed Etoketim, Mamadou Bah, Bennoit Assogba, Umberto D'Alessandro

Medical Research Council Unit The Gambia at the London School of Hygiene & Tropical Medicine, Banjul, Gambia

Seasonal malaria chemoprevention (SMC), the administration of sulfadoxine -pyrimethamine (SP) plus amodiaquine (AQ) to children 3 months to < 5 years in the community, is being implemented in the Sahel to reduce malaria morbidity. SP and AQ lead to increase in gametocytes, the transmissible stage of malaria, when administered in clinical *P falciparum* malaria. It is debated if SMC would lead to increased gametocytes and thus transmission when administered in asymptomatic infections. We enrolled children aged 2 to < 5 years hence eligible for the SMC intervention (SMC group) and a control group aged 5 to 8 years who are ineligible in 2017 and 2018 in eastern Gambia. Children were assessed 4 days before (baseline) and then 14 days after (endline) the first cycle of the SMC intervention. All had asymptomatic *P falciparum* infection determined by polymerase chain reaction (PCR) at enrolment. Gametocytes were measured by quantitative nucleic acid sequence based amplification assay (QT-NASBA) at baseline and at endline. Primary objective was to determine the effect of SMC intervention on gametocytes prevalence and density. We compared gametocyte prevalence between groups using chi-squared test and assessed within-group change in gametocyte density using Wilcoxon signed rank test on log-transformed densities. 65 children in the SMC group and 75 in the control group were enrolled. Baseline gametocytes prevalence was 10.7% and 13.3% for the SMC and control groups respectively (p 0.64) and was 9.4% and 12.6% in the SMC and control groups respectively at endline (p 0.57). For within groups comparison (baseline vs endline), there was a reduced odds of gametocyte carriage at endline in both groups: SMC group (OR 0.6, 95% CI 0.14 - 2.51, p 0.48) and the control group (OR 0.9, 95% CI 0.34 - 2.30, p 0.80) but was not statistically significant. Similarly, there was no statistically significant change in gametocyte density in both the SMC (p 0.62) and in the control group (p 0.59). In our low transmission setting where most asymptomatic infections are at low densities, SMC intervention did not increase gametocytes prevalence or density in asymptomatic *P falciparum* carriers.

1156

MALARIA PREVALENCE AND INCIDENCE IN HIV POSITIVE, SEROCONVERTED, AND HIV NEGATIVE INDIVIDUALS IN A HOLOENDEMIC SETTING IN KISUMU COUNTY, WESTERN KENYA

Risper Maisiba[1], Benjamin Opot[1], Raphael O. Okoth[1], Farid Abdi[1], Maureen Mwalo[1], Jew Ochola[2], June Otieno[3], Eric Ogonda[1], Dennis Juma[1], Valentine Singoei[2], Hosea Akalah[1], John Owuoth[2], Ben Andagalu[1], Amanda L. Roth[1], Julie Ake[4], Christina Polyak[4], Edwin Kamau[4]

[1]*U.S. Army Medical Research Directorate-Africa, Nairobi, Kenya,* [2]*HJF Medical Research International/U.S. Army Medical Research Directorate – Africa, Kisumu, Kenya,* [3]*U.S. Army Medical Research Directorate-Africa, Kisumu, Kenya,* [4]*U.S. Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, MD, United States*

HIV and malaria tend to interact bi-directionally and synergistically, affecting clinical outcome and patient management. We investigated the prevalence of *Plasmodium* species infections stratified on HIV status. We analyzed data from a prospective observational HIV incidence cohort study in Kombewa, Kenya. Study participants were clinically healthy, HIV-seronegative and seropositive adults aged 18-35 years. Participants were followed for 24 months, with blood samples collected quarterly for several laboratory tests. Malaria diagnosis was performed by microscopy and PCR. Initial PCR detected *Plasmodium*, which was then speciated for *P. falciparum* (Pf), *P. malariae* (Pm), *P. ovale curtisi* (Poc), and *P. ovale wallikeri* (Pow). Standard statistical analyses were applied. Samples (n=1034) collected February 2017 to March 2020 from 121 study participants were analyzed. Microscopy readouts detected *Plasmodium* in 31.0%, 18.0% and 20.0% in HIV-uninfected, seroconverters and HIV-infected individuals, respectively. By PCR, *Plasmodium* was detected in 50.0%, 13.0% and 12.4% in the above groups. The frequency of Pf among the above groups was 22.3%, 4.4% and 6.9%, respectively. The frequency of Pm was 8.1% and 1.3% in HIV-uninfected and HIV-infected individuals, respectively. The frequency of Poc was 2.5% and 0.9%, and Pow was 1.0% and 1.8%, in HIV-uninfected and HIV-infected individuals, respectively. The adjusted risk of being parasitemic was 2.4 (95% CI 2.1-2.7), 0.5 (95% CI, 0.3-0.9) and 0.5 (95% CI, 0.4-0.6) in HIV-uninfected, seroconverters and HIV-infected individuals, respectively. The relative risk of *Plasmodium* in HIV-uninfected individuals was 4.5 times that of HIV-infected and seroconverters individuals combined (RR=4.5; p<t0.001). 68.9% of HIV-uninfected individuals had submicroscopic *Plasmodium* infections compared to 30.3% of HIV-infected (p=0.006). The findings show high prevalence of patent

and sub-patent parasitemia in the HIV-uninfected individuals. For non-falciparum malaria, Pm was at higher frequency in HIV-uninfected but not in HIV-infected. Additional analyses are underway.

1157

DATA-DRIVEN AND INTERVENTION-SPECIFIC STRATIFICATION IN MOZAMBIQUE TO GUIDE DECISION MAKING IN A HIGH-BURDEN, HIGH-IMPACT COUNTRY

Baltazar Candrinho[1], Guidion Mathe[1], Rita Chico[2], Bradley Didier[3], James Colborn[4]

[1]*Mozambique National Malaria Control Program, Maputo, Mozambique,* [2]*Clinton Health Access Iniative, Maputo, Mozambique,* [3]*Clinton Health Access Initiative, Boston, MA, United States,* [4]*Clinton Health Access Initiative, Maputo, Mozambique*

WHO's High-Burden/High Impact (HBHI) initiative was created with the idea that data-driven decision making, taking into account all relevant information on malaria transmission in a given area, can have significant impacts on global malaria transmission if properly targeted to the highest burden areas and countries. Mozambique is among the HBHI countries, with heterogeneity ranging from very high burden provinces with prevalence >60%, to very low burden provinces with prevalence <1% and elimination goals. Given this heterogeneity, data-driven stratification of the country has been vital for general planning purposes, though use of this stratification for implementation and expansion of interventions has been limited due to the generalized nature of the methodology. Here we describe an intervention-specific approach to stratification in order to guide prioritization of implementation interventions, based on a combination of burden (estimated from modeled incidence), access to care, treatment seeking behavior, seasonality, mortality, and predicted modeled impact on transmission. Districts were ranked according to the sets of indicators appropriate for the following interventions: seasonal malaria chemoprophylaxis (SMC), intermittent preventive treatment in infants or schoolchildren (IPTi/IPTsc), indoor residual spraying (IRS), and surveillance. Final lists of ranked districts for each intervention were provided to the National Malaria Control Program for decision making on future implementation or expansion of interventions, as well as for prioritization and reinforcement of existing partner activities. Intervention-specific stratification, using data relevant to each intervention, has the potential to focus limited resources to areas where they will have the greatest impact on malaria transmission. This approach is particularly relevant in countries such as Mozambique that have highly heterogenous malaria transmission.

1158

INTEGRATING PARASITOLOGICAL AND ENTOMOLOGICAL OBSERVATIONS TO UNDERSTAND MALARIA TRANSMISSION IN RIVERINE VILLAGES IN THE PERUVIAN AMAZON

Angel Rosas-Aguirre[1], Marta Moreno[2], Diamantina Moreno-Gutierrez[3], Alejandro Llanos-Cuentas[4], Marlon Saavedra[5], Juan Contreras-Mancilla[5], Jose Barbosa[5], Freddy Alava[5], Kristhian Aguirre[4], Gabriel Carrasco-Escobar[6], Catharine Prussing[7], Joseph Vinetz[8], Jan E. Conn[7], Niko Speybroeck[9], Dionicia Gamboa[5]

[1]*Fund for Scientific Research FNRS, Brussels, Belgium,* [2]*London School of Hygiene & Tropical Medicine, Department of Infection Biology, London, United Kingdom,* [3]*Facultad de Medicina Humana, Universidad Nacional de la Amazonía Peruana, Iquitos, Peru,* [4]*Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru,* [5]*Laboratorio ICEMR-Amazonía, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru,* [6]*Division of Infectious Diseases, Department of Medicine, University of California San Diego, La Jolla, CA, United States,* [7]*Wadsworth Center, NYSDOH, Albany, NY, US; School of Public Health, Department of Biomedical Sciences, Albany, NY, United States,* [8]*Section of Infectious Diseases, Department of Internal Medicine, Yale School of Medicine, New Haven, CT, United States,* [9]*Research Institute of Health and Society (IRSS), Université catholique de Louvain, Brussels, Belgium*

Rural, remote riverine villages account for most of the reported malaria cases in the Peruvian Amazon. As transmission decreases due to intensive standard control efforts, malaria strategies in these villages will need to be more focalized and adapted to the local epidemiology. By integrating parasitological, entomological and environmental observations between January 2016 and June 2017, we provided an in-depth characterization of the malaria transmission dynamics in four riverine villages of Mazan district, Loreto department. Despite variations across villages, malaria prevalence by PCR in March 2016 was high (>25% in three villages), caused by *P. vivax* mainly and composed of mostly submicroscopic infections. Housing without complete walls was the main malaria risk factor, while households close to forest edges were more commonly identified as spatial clusters of malaria prevalence. Villages in the basin of the Mazan River had a higher density of adult *Anopheles darlingi* mosquitoes and retained higher prevalence and incidence rates compared with villages in the basin of Napo River despite test-and-treat interventions. High heterogeneity in malaria transmission was found across and within riverine villages, resulting from interactions between the micro-geographic landscape driving diverse conditions for vector development, housing structure, and human behaviour.

1159

HIGH PREVALENCE OF CO-INFECTION IN PATIENTS WITH MALARIA: A CROSS-SECTIONAL STUDY IN THE MAIN ENDEMIC REGION OF VENEZUELA

David A. Forero-Peña[1], Marisol Sandoval De Mora[2], Ivan Amaya[3], Angel Gamardo[1], Melynar Chavero[1], Luisamy Figuera[1], Natasha A. Camejo-Avila[1], Maria V. Marcano[1], Mariana Hidalgo[4], Cariagne J. Arenas[1], Myriam A. -Herrera[5], Socrates Herrera[5]

[1]*Biomedical Research and Therapeutic Vaccines Institute., Ciudad Bolivar Venezuela, Bolivarian Republic of Venezuela,* [2]*Department of Internal Medicine, Ruiz y Páez University Hospital Complex., Ciudad Bolivar, Bolivarian Republic of Venezuela,* [3]*Department of Parasitology and Microbiology, School of Health Sciences, Universidad de Oriente, Núcleo Bolívar, Ciudad Bolivar, Bolivarian Republic of Venezuela,* [4]*Immunoparasitology Laboratory, Center for Microbiology and Cell Biology, Venezuelan Institute for Scientific Research., Caracas, Bolivarian Republic of Venezuela,* [5]*Caucaseco Scientific Research Center, Cali, Colombia, Colombia*

Malaria continues to be a major public health problem worldwide. Simultaneous infections with more than one pathogen not only complicate the diagnosis, but they can also change the clinical course, the similarities in clinical presentation of malaria and the superimposed endemicity, can result in a sub-diagnosis of a co-infection, which could lead to increased mortality for this disease. Venezuela, has no studies that describe the presence of co-infections in patients with malaria. Between June and November 2018, a cross-sectional study was conducted, including patients who went to any of the three reference medical centers in Ciudad Bolívar, Bolívar state and who were positive for *Plasmodium spp*. infection by thick drop and spread of blood. A clinical and laboratory evaluation of each patient was performed. Coinfections with Dengue (DEV), Viral Hepatitis (VH) (A, B and C), Leptospirosis (LEP), and Chikungunya (CHIKV) were evaluated using the ELISA technique. A total of 161 patients were studied, 106 (65.8%) presented *P. vivax* infection, 43 (26.7%) *P. falciparum* and 12 (7.4%) had mixed malaria (*P.f /P.v*). Co-infection was found in 55/161 (34.2%) patients and was more frequent in patients with *P. falciparum* (n = 21/43, 48.8%) (OR= 2,43; 95%CI= 1.39-4.25; P= 0,02) compared with *P. vivax* (n = 23/106, 38.2%) and *P.v/P.f* (n = 3/12, 25%). The most prevalent coinfection was with DENV (14.9%), followed by HAV (11.8%), HBV (6.2%), CHIKV (5.5%) and LEP (3.7%). Complicated malaria was significantly more frequent in the co-infected group than in the not co-infected group (56.4% Vs. 35.8%; OR: 2,31; 95%CI= 1.18-4.92; P= 0.01). According to our serological studies, there is a high prevalence of

co-infections in patients with malaria in this region, and it was related to a worse outcome. Prospective studies with samples at different points of infection and the use of molecular tools are needed to clarify these findings.

1160

ANTIBODY PROFILE KINETICS TO *PLASMODIUM FALCIPARUM* DURING A PERIOD OF DECLINING MALARIA TRANSMISSION IN SOUTHERN ZAMBIA FROM 2008 TO 2015

Tamaki Kobayashi[1], Sophie Berube[1], Harry Hamapumbu[2], Philip E. Thuma[2], Lindsey Wu[3], Kevin Tetteh[3], Chris Chris Drakeley[3], Thomas A. Louis[1], William J. Moss[1]

[1]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [2]*Macha Research Trust, Choma, Zambia,* [3]*London School of Hygiene & Tropical Medicine, London, United Kingdom*

Extensive research has been done on antibody responses to *Plasmodium falciparum* in understanding their role in protective immunity and as a measure of recent and past exposure to the parasite. However, limited information is available on the kinetics of the antibody response in a low transmission setting. In the Macha Hospital catchment area in Southern Province, Zambia, malaria transmission declined dramatically over the past two decades. Formerly mesoendemic with transmission peaking during the rainy season, the area is now approaching malaria elimination. We collected dried blood samples (DBS) from 140 individuals (median age at baseline = 14 years, IQR = 6.3, 27) approximately every other month from 2008 to 2015. Over this period, parasite prevalence by rapid diagnostic test (RDT) among cohort participants decreased from 4.0% to 0.0%. Plasma was extracted from DBS and a multiplex bead assay measured IgG antibody levels among the sampled individuals. The bead assay is comprised of 20 antigens, including 19 *P. falciparum* antigens. The longitudinal samples in this study provide information on the baseline immunological profiles of individuals in a low transmission region as well as temporal variations in antibody response to declining *P. falciparum* transmission. Together, these data will give us insight into the kinetics of antibody responses to falciparum malaria in a near-elimination region.

1161

PROGNOSTICS OF MALNUTRITIONAL STATUS IN THE OCCURRENCE OF MULTIPLES MALARIA EPISODES IN CHILDREN AGED 6-59 MONTHS FROM 2013-2016 IN DANGASSA, KATI HEALTH DISTRICT MALI

Oumar Thiero[1], Soumba Keita[2], Aissata Sacko[2], Kola Cusse[2], Seydou Doumbia[2]

[1]*Tulane University, USTTB, FMOS, DER Public Health, UCRC, New Orleans, LA, United States,* [2]*USTTB, FMOS, UCRC, Bamako, Mali*

The relationship between nutritional status and malaria remains complex and difficult to interpret. Anemia and underweight have often been described as risk factors for multiples malaria episodes. Objective: We accessed the relationship between nutritional status and multiples-malaria-episodes in children aged 6-59-months from 2013-2016 in Dangassa, Kati health district of Mali. Method: A longitudinal community-based study was performed with cross-sectional-survey at the beginning and at the end of malaria-transmission-period for baseline and the active passive-case-detection. The repeated ordinal-logistic-model using GEE-with-maximum-likelihood-method and the exposure-time as the offset was used to estimate the odd-ratio for having higher multiples-malaria-episodes. In 2013, 2014, 2015 and-2016, respectively 199, 211,151 and 312 children were enrolled at the beginning of transmission-season (May/June). The Malaria prevalence was respectively 15.6-11.4-8.6-and-6,9. Negative children was selected and followed for malaria episodes over the-transmission-season. The end of the season the same procedure was applied over the dry season. Over transmission period, children without anemia were 1.9 times more likely (OR=1.851-p=0.013) to have higher multiple malaria episodes compared to children with anemia. The number of underweight children with higher multiple malaria episodes increased by 14.1% compared to no-underweight children, but not significant(OR=1.141-p=0.641).Children aged 36-59 months was 1.85 times higher risk of having higher multiples-episodes compared to children 6-24-months(OR=1.851-p=0.016). There was no difference between the ref and 24-36-months(p=0.114). The risk of having higher multiple malaria episodes was decreasing significantly for the years 2014-2015-and-2016 compare to 2013(p=0.000). This decrease maybe explained by the implementation of the seasonal-malaria-chemoprophylaxis during the study period. Conclusion:Our study showed that multiples malaria episodes are significantly related to the nutritional status among children during the-transmission-season.

1162

DEEPA PINDOLIA ABSTRACT

Cheick Saïd Compaore[1], James-Francis Onohiol[2], Thibaud de Chevigny[3], Jeanne Umuhire[3], Emily Crawford[4], Deepa Kishor Pindolia[5], Arnaud LeMenach[6], **Gauthier Tougri**[1]

[1]*National Malaria Control Program, Ouagadougou, Burkina Faso,* [2]*CHAI, Ouagadougou, Burkina Faso,* [3]*CHAI, Dakar, Senegal,* [4]*CHAI, Abuja, Nigeria,* [5]*CHAI, Nairobi, Kenya,* [6]*CHAI, Washington, DC, United States*

In 2018, the Burkina Faso Health Management Information System was notified of approximately 12 million malaria cases. As malaria surveillance is a core intervention, in 2020, the National Malaria Control Program evaluated the strengths and weaknesses of its malaria surveillance systems to inform future activities. An observational study consisting of a desk review, semi-structured interviews at central and sub-national level, structured interviews with health workers (n=176) and community health workers (n=108), and an assessment of HMIS data quality were conducted nationally in 2020 in public, formal private, and community sectors. Malaria-related surveillance systems (cases, commodities, entomology, and interventions) were evaluated based on data use, data quality, and representativeness of the systems. Gaps were explained based on contextual, infrastructural, process, technical and behavioral determinants. Surveillance systems cover the whole country, include all key sectors, and collect case, vector, intervention and commodities data. However, each system flows in separate databases with no comprehensive data integration. Between 2017 and 2020, 90% of health facilities reported into the HMIS at least once, but reporting rates varied between 53% and 80%. Case data (via a dashboard) and epidemiological bulletins are available at central and subnational levels. Information is used at the central level to prioritize interventions, request funding, and inform commodity distribution. Integrating surveillance data from all systems into a data repository, increasing data quality, and enabling health facility access to data would facilitate evidence-based decision making for malaria control. Improved surveillance systems will be essential to support Burkina Faso goal of reducing malaria mortality by 75% by 2025.

1163

SELECTION AND UTILITY OF SINGLE NUCLEOTIDE POLYMORPHISM MARKERS TO REVEAL FINE-SCALE POPULATION STRUCTURE IN *PLASMODIUM VIVAX*

Anthony Ford[1], Daniel Kepple[2], Beka Abagero[3], Sarah Auburn[4], Sisay Getachew[5], Andargie Abate[6], Lemu Golassa[7], Guiyun Yan[8], Delenasaw Yewhalaw[3], Eugenia Lo[2]

[1]*Department of Mathematics and Statistics, University of North Carolina at Charlotte, Charlotte, NC, United States,* [2]*Department of Biological Sciences, University of North Carolina at Charlotte, Charlotte, NC, United States,* [3]*Tropical Infectious Disease Research Center, Jimma University, Jimma, Ethiopia,* [4]*Global and Tropical Health Division of Menzies School of Health Research, Darwin, NT, Australia,* [5]*Armauer Hansen Research Institute, College of Natural Sciences at Addis Ababa University, Addis Ababa, Ethiopia,* [6]*Bahir Dar University, Bahir Dar, Ethiopia,* [7]*Medical*

Parasitology Research Unit, Aklilu Lemma Institute of Pathobiology (ALIPB), Addis Ababa University, Addis Ababa, Ethiopia, [8]Program in Public Health, University of California at Irvine, Irvine, CA, United States

Single nucleotide polymorphisms (SNPs) have been shown to be useful in revealing population structure at the continental and regional levels. A careful selection of high performance (HP) SNP markers would allow us to track the spread of the disease in local communities and provide an important addition to traditional disease surveillance especially for high-risk populations. Recently, there has been a substantial increase in *Plasmodium vivax* cases across Ethiopia hinting that potential human movements and agricultural activities may have facilitated the spread of infection across and within communities. In this study, we aim to identify a panel of HP SNPs to analyze fine-scale population structure of *P. vivax* in endemic areas in Ethiopia, using 44 *P. vivax* whole genome sequences representing different parts of Southwestern Ethiopia. We restricted our focus to synonymous SNPs located within the core of the 14 chromosomes independent of hypervariable and subtelomeric regions. Based on preliminary analyses, a total of 277 high-quality synonymous SNPs on chromosome 9 and an additional 65 on chromosome 10 were first identified from the 44 genomes. A smaller set of SNP candidates was then selected from this initial SNP panel based on their performance for individual assignment (BELS ranking), the level of polymorphisms (minor allelic frequency), and genetic differentiation among the study sites (pairwise F_{ST} values). We validated the resolution and assignment power of the selected HP SNPs using multivariate principal component and Bayesian analysis to reveal the clustering patterns among individuals. Further investigation is needed to determine if these HP SNPs are useful in tracing the spread of *P. vivax* with expanded samples and at broader geographical scales, particularly concerning the existence of admixed populations of Duffy-positive and Duffy-negative individuals in Ethiopia.

1164

MOLECULAR EPIDEMIOLOGY OF *PLASMODIUM FALCIPARUM* BY MULTIPLEXED AMPLICON DEEP SEQUENCING IN SENEGAL

Tolla Ndiaye

Cheikh Anta Diop University, Dakar, Senegal

Molecular epidemiology can provide important information regarding the genetic diversity and transmission dynamics of *Plasmodium falciparum*, which can assist in designing and monitoring elimination efforts. Next Generation Sequencing, NGS, offers a practical, fast, and high-throughput approach to understand malaria population genetics. This study aimed to unravel the population structure of *P. falciparum* and to estimate the allelic diversity, multiplicity of infection (MOI), and evolutionary patterns of the malaria parasite using the NGS platform. Multiplex amplicon deep sequencing was carried out for the highly polymorphic regions of merozoite surface protein 1 (*Pfmsp1*) and merozoite surface protein 2 (*Pfmsp2*) genes in *P. falciparum* for 53 isolates from two epidemiologically distinct areas in the South (high malaria transmission) and North (low malaria transmission) of Senegal. A total of 76 *Pfmsp1* and 116 *Pfmsp2* clones were identified and 135 different alleles types were found, of which 56 belonged to the *Pfmsp1* gene and 79 to the *Pfmsp2* gene. K1 and IC3D7 allelic families were most predominant in the two study sites. The local Heterozygosity and nucleotide diversity (ϖ) were higher in the South than in the North for both genes. For *Pfmsp1*, a high positive Tajima's D (TD) value was observed in the South (D=2.0453) while negative TD value was recorded in the North (D=-1.46045) and F-Statistic (Fst) was 0.19505. For *Pfmsp2*, non-directional selection was found with a highly positive TD test in both areas and Fst was 0.02111. The mean MOI for both genes was 3.07 and 1.76 for the South and the North, respectively, with a statistically significant difference between areas (p=0.001). Multiplexed amplicon deep sequencing allowed sequencing several genes of interest simultaneously and can be readily adapted to other genes as the sequencing of antimalarial resistance markers. This study also revealed an almost panmictic *P. falciparum* population which seems caused by important gene flow. Further studies, including modelling, are necessary to assess the true dynamics of malaria transmission within Senegal.

1165

GENETIC DIVERSITY OF *PLASMODIUM FALCIPARUM* STRAINS IN YAOUNDE, CAMEROON AMONG PATIENTS SUFFERING FROM UNCOMPLICATED MALARIA FOR 2014 AND 2018

Selly Ngaloumo Abdel Aziz, Akindeh Mbuh Nji, Peter Thelma Niba, Aristid Ekollo Mbange, Calvino Tah Fomboh, Jean Paul Chedjou, Wilfred Fon Mbacham

University of Yaounde 1, Yaounde, Cameroon

Massive population migration is a major determinant that may affect the transmission dynamic of malaria. Importantly, this can fuel and increase the genetic complexity of parasites, inducing multiple drug resistance to antimalarial drug combinations. This study sought to analyze the genetic diversity of *Plasmodium falciparum* among patients suffering from uncomplicated malaria in Yaoundé for 2014 and 2018. Patients who sought for malaria treatment in Cite-Verte district hospital and Etoug-Ebe Baptist hospital, Yaoundé were recruited. The Random amplified polymorphic DNA (RAPD)-PCR was employed with four arbitrary single primers (E4, E8, L12, R8), whereas nested-PCR was also used that targets the msp2 region. Analysis of size polymorphism for the msp2 and RAPD PCR products was performed by agarose gel electrophoresis on a 2.0% and 1.5% gel concentration, respectively. The Phyltool software was used to generate a distance matrix from which a genetic distance tree was inferred on MEGA software ver. 10. Data were analyzed using SPSS ver 20. Calculation of the mean genetic distance using the msp2 marker revealed that isolates collected in 2014 exhibited significantly greater genetic diversity than those collected in 2018 (0.93±0.16 vs 0.91±0.17; P=0.004). Similarly, the multiplicity of infection (MOI) was higher in 2014 (MOI=1.84) than in 2018 (MOI=1.73). Of the 4 RAPD primers employed E8 was the most discriminatory. The genetic distance tree based on this primer was thus used to infer phylogenetic relationships amongst parasites strains. Globally, the sub-clusters showed intermixed isolates from the 2 time-points. However, 3 sub-sub-clusters could be identified with almost exclusively isolates collected in 2014. Two sub-sub-clusters could also be identified that were specific to 2014. The msp2 and E8 markers performed equally in defining the mean genetic distance amongst *Plasmodium falciparum* populations (Bonferroni test; P=1.00). Despite population migrations, the level of *Plasmodium falciparum* diversity has remained low in 2018. The parasites populations isolated in 2014 and 2018 were, in general, phylogenetically related.

1166

EFFICACY AND SAFETY OF ARTEMISININBASED COMBINATION THERAPY AND THE IMPLICATIONS OF *PFKELCH13* AND *PFCORONIN* MOLECULAR MARKERS IN TREATMENT FAILURE IN SENEGAL

Mamadou Samb YADE

Cheikh Anta Diop University, Dakar, Senegal

Afterthe withdrawal of chloroquine in 2006, Senegal switched to artemisinin-based combination therapy (ACT) as first-line treatment to manage uncomplicated malaria. Monitoring therapeutic efficacy of antimalarials is essential for ensuring appropriate use of ACTs. This study aimed to update the status of ACT antimalarial efficacy ten years after their first introduction in Senegal. This was a randomized, three-arm, open-label study to evaluate the efficacy and safety of artemether-lumefantrine (AL), artesunate-amodiaquine (ASAQ), and dihydroartemisinin-piperaquine (DP). The study was conducted in Kedougou region (high malaria transmission) and Diourbel region (moderate transmission).Patients presenting with uncomplicated *Plasmodium falciparum* malaria were screened, enrolled, treated, and followed for 28 days for AL and ASAQ arms or 42 days for DP arm. Clinical and parasitological responses were assessed following antimalarial

treatment. Genotyping (*msp1*, *msp2* and 24 SNP barcode) was done to differentiate recrudescence from re-infection. Samples with PCR-confirmed treatment failure were evaluated for *P. falciparum kelch 13(Pfk13)*propeller (codons 450 - 680) and *P. falciparum coronin (Pfcoronin)* genes (codons 31 - 186). Data entry and analysis were done using the WHO designed Excel-based application. Of the 496 patients enrolled, 199, 200 and 97 were assigned to AL, ASAQ and DP arms respectively. For AL and ASAQ arms, 185 out of 199, and 191 out 200, respectively, completed the follow-up schedule, while for the DP arm, 85 out of 97 completed follow-up. In Diourbel, PCR non-corrected ACPR at day-28 was 100.0% (95%CI: 96.3-100.3) in both the AL and ASAQ arms. In Kedougou, PCR non-corrected ACPR were 96.5%, 100% and 96.4% in AL, ASAQ and DP arms respectively and when PCR-corrected, ACPR values were 98.8%, 100% and 97.6%. No*Pfk13*or *Pfcoronin*mutations associated with artemisinin resistance were found in analyzed samples. This study shows that AL, ASAQ and DP remain efficacious and well-tolerated in the treatment of uncomplicated *P. falciparum* malaria in Senegal.

1167

OPTIMIZATION OF MCFACSSEQ FOR LARGE SCALE TRANSCRIPTOME PROFILING OF *PLASMODIUM FALCIPARUM* ISOLATES

Catherine Jett, Aliou Dia, Xue Li, Anna Reyes, Marina McDew-White, **Ian H. Cheeseman**, Timothy J. Anderson

Texas Biomedical Research Institute, San Antonio, TX, United States

Transcriptome sequencing provides a detailed picture of how RNA levels differ between samples. The application of transcriptomics to malaria parasites is constrained by the need for large, synchronous cultures. We previously validated an approach to capture the transcriptome of the intraerythrocytic development cycle (IDC) of *Plasmodium falciparum* at high resolution (mcFACSseq) which combines flow sorting with low input RNA sequencing to assay the complete IDC from an asynchronous culture. We sought to improve throughput, coverage and accuracy and decrease costs associated with high numbers of isolates to make better use of this valuable tool. We improved resolution at early life cycle stages through changes to the gating strategy decreasing sort times by ~30%. By pooling of amplified cDNA samples, introducing barcoded unique molecular identifiers before library production, and using tagmentation to produce sequencing libraries we improved processing efficiency and expression level quantification accuracy. We found that adding bovine serum albumin during cell lysis and additional QC allowed us to avoid samples with poor quality cDNA and reduce the number of amplification cycles from 30 to 22, decreasing bias and increasing transcriptome coverage. We validated our 3' targeted approach by sampling a panel of isolates and found that while the total number of genes detected decreased compared to the full-length cDNA protocol in mcFACSseq, replication of expression levels in the identified genes was significantly increased and differential expression could be robustly determined.

1168

INFECTION DYNAMICS OF PLASMODIUM FALCIPARUM USING AMPLICON DEEP SEQUENCING DURING AN ACT EFFICACY TRIAL IN NW ETHIOPIA

Daniel Castaneda-Mogollon[1], Habtie Tesfa[2], Aberham Abere[2], Abebe Genetu Bayih[2], Ranmalee Amarasekara[1], Conrad Izydorczyk[1], Dylan R. Pillai[1]

[1]*University of Calgary, Calgary, AB, Canada,* [2]*University of Gondar, Gondar, Ethiopia*

According to the WHO, almost two-thirds of the Ethiopian population are at risk of contracting malaria, where *Plasmodium falciparum* accounts for 60% of the cases. We previously showed that mutations in the propeller domain of the *kelch13 (K13)* are present in NW Ethiopia but not associated with delayed parasite clearance. We analyzed the infection dynamics of malaria by looking at 97 *P. falciparum* PCR-confirmed individuals in the region of Gondar, Ethiopia, during a 28-day *in vivo* efficacy artemisinin combination therapy (ACT). We compared the genotypes and complexity of infection (COI) results by employing PCR-agarose gel and amplicon deep sequencing of the gene markers *(ama1)* and the conserved *Plasmodium* membrane protein *(cpmp)*. We sequenced the *kelch13* gene on those individuals whose parasite density have a lower rate of molecular clearance after being treated with ACT, in order to assess the presence of unique SNPs that could be associated with this phenotype. The results of deep sequencing, traditional genotyping and phenotypic data will be presented for 97 patients. Understanding the infection mechanisms of *P. falciparum* in this region could provide a better insight of the geographic extension of artemisinin tolerant clones and its implications in public health. Furthermore, clone dominancy variation over time may explain why some individuals have different kinetics of clearance. Nevertheless, additional genotyping studies are required to assess the surveillance of artemisinin-resistant *Plasmodium* species across the region.

1169

TEMPORAL GENETIC VARIATION OF *PLASMODIUM FALCIPARUM* PARASITES FOLLOWING THE IMPLEMENTATION OF ARTEMISININ-BASED THERAPIES IN THE VILLAGE OF FALADJÉ IN MALI

Fatoumata Maiga[1], Antoine Dara[1], Jeffrey Shaffer[2], Jian Li[2], Cheickna Cisse[1], Wele Mamadou[1], Abdoulaye Djimdé[3]

[1]*African Center of Excellence in Bioinformatics in Bamako, University of Sciences Technics and Technologies of Bamako, Bamako, Mali,* [2]*Tulane University, New Orleans, LA, United States,* [3]*Molecular Epidemiology and Drug Resistance Unit, University of Sciences Technics and Technologies of Bamako, Bamako, Mali*

Malaria remains endemic in Mali despite of intensification of activities since ACTs introduction in 2007. The persistence of vector transmission may be due to several factors including the genetic variability of *Plasmodium falciparum* that allows it to adapt to its hosts. Thus understanding *Plasmodium falciparum* population variations before and after ACTs introduction could provide new strategies in control malaria in Mali. In this work, we studied temporal genetic variation in *Plasmodium falciparum* populations sampled in Faladje, a malaria hyper-endemic area where efficacy studies on artemisinin have been conducted. A total of 449 whole genome sequences of *Plasmodium falciparum* isolates collected at Faladje have been analyzed using bioinformatics tools for determining the genetic diversity and the multiplicity of infections in a repeated retrospective cross-sectional study over a 10-year period before and after the ACTs introduction (2007-2017). We expect that genetic variability of *Plasmodium falciparum* populations may be affected by the introduction of ACTs in the region, which subsequently may result in infection multiplicity. Characterization of genetic diversity of *Plasmodium falciparum* populations in Faladje will be provided and will contribute to the monitoring of this disease.

1170

ASSOCIATION BETWEEN *FCGR3A* RS396991 AND *FCGR3B* RS5030738 POLYMORPHISMS AND *PLASMODIUM FALCIPARUM* INFECTION OUTCOME IN GHANAIAN CHILDREN

Quaneeta Mohktar[1], Daniel Dodoo[1], Michael Theisen[2], Bright Adu[1]

[1]*Noguchi Memorial Institute for Medical Research, Accra, Ghana,* [2]*Centre for Medical Parasitology at Department of International Health, Immunology, and Microbiology and Department of Infectious Diseases, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark*

Immunity to *Plasmodium falciparum* malaria develops after prolonged exposure to the parasite and is thought to be mediated through binding of specific immunoglobulin (Ig) G antibodies to Fc gamma receptors. Polymorphisms in *FCGR3A* and *FCGR3B* affect their binding affinities to IgG subclasses. We previously showed that *FCGR3B* polymorphisms modify the protective effect of malaria-specific antibodies. Here, we studied

associations between *FCGR3A* and *FCGR3B* haplotype and the risk of febrile malaria in Ghanaian children. *FCGR3A* and *FCGR3B* genotypes of children (n =121; aged 1-12 years) living in a malaria endemic community who were part of a 42-week longitudinal cohort study was obtained by multiplex polymerase chain reaction and sequencing, respectively. Associations with either protection or susceptibility to malaria was assessed for each genotype and their haplotypes. The A-allele of *FCGR3B*-c.233C>A (rs5030738) (additive model: odds ratio (OR)=0.49, 95% confidence interval (CI)=0.25-0.95, p=0.03), the G-allele of *FCGR3A*-c.558T>G (rs396991) [over dominant model: OR=0.36, 95% CI=0.16-0.79] were significantly associated with protection against febrile malaria respectively. Similarly, the TA haplotype was significantly associated with protection against febrile malaria (OR=0.33, 95% CI=0.13-0.85, p=0.022) in the cohort. Our results call for a more detailed mechanistic assessment of the potential effect modifications of malaria protective antibodies by these two polymorphisms and their haplotypes.

1171

DISPERSION PATTERN OF *PLASMODIUM VIVAX* AMONG FIVE DIFFERENT SETTINGS IN THE PERUVIAN AMAZON

Lidia E. Llacsahuanga Allcca[1], Paulo Manrique[2], Christopher Delgado-Ratto[3], Juan Contreras-Mancilla[1], Jose Barbosa[1], Niko Speybroeck[4], Joseph Vinetz[5], Alejandro Llanos-Cuentas[2], Angel Rosas-Aguirre[4], Dionicia Gamboa[1]

[1]*Laboratorio ICEMR-Amazonía, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru,* [2]*Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru,* [3]*Global Health Institute, University of Antwerp, Antwerp, Belgium,* [4]*Research Institute of Health and Society (IRSS), Université catholique de Louvain, Brussels, Belgium,* [5]*Section of Infectious Diseases, Department of Internal Medicine, Yale School of Medicine, New Haven, CT, United States*

As malaria transmission decreases in Peru following intensive control efforts, *Plasmodium vivax* becomes a major challenge to move towards malaria elimination. In addition to the dormant hypnozoite stage and the large proportion of asymptomatic-submicroscopic infections, the local human mobility shapes a complex distribution and dispersion pattern which hinders malaria interventions. Consequently, decision-makers need a comprehensive understanding of *P. vivax* transmission dynamics in order to develop and apply focalized interventions. In that sense, genetic analysis can contribute to elucidate the transmission dynamics of parasite populations, usually hidden to other approaches. In this study, we genotyped *P. vivax* parasites found in 950 blood samples collected in five different river basins in the Peruvian Amazon: Napo, Mazan, Nanay, Itaya and Tigre. Bayesian modelling was used to determine the magnitude and directionality of parasites' gene flow among those populations. The overall genetic diversity (*Hexp*) in study areas was 0.66; Mazan presented the highest diversity (*Hexp*=0.71) and Napo the lowest one (*Hexp*=0.39). Parasites found in Napo were the most genetically differentiated to the peers found in other areas (JostD between 0.24 and 0.36) and four unevenly distributed genetic clusters were predicted. Gene flow analysis revealed an asymmetrical dispersion pattern and strong divergence rates in accordance with a steppingstone model of gene flow. Temporal gene flow analysis also showed a consistent impact of divergence and unidirectional patterns of dispersion. The high divergence rates (*D*=18.4-23.9) and ongoing gene flow across study areas indicate a lagged but persistent impact of human mobility in establishing malaria diversity within these areas. All in all, these results support the hypothesis that *P. vivax* transmission persists through malaria "corridors" driven by human movement in the Peruvian Amazon. Moreover, the routes of dispersion here described are important for the formulation of future control policies.

1172

PLASMODIUM FALCIPARUM GENETIC VARIATIONS UNDERLYING ACQUISITION OF MALARIA INFECTIONS AMONG GHANAIAN CHILDREN

Selassie Majola Sawyerr Kumordjie[1], Quratul-ain Issahaque[1], Tracy Sarkodie-Addo[1], Fareed Arthur[2], Jeff Bailey[3], Bright Adu[1], Daniel Dodoo[1]

[1]*Noguchi Memorial Institute, Accra, Ghana,* [2]*Kwame Nkrumah University of Science and Technology, Kumasi, Ghana,* [3]*Brown University, Providence, RI, United States*

Factors determining the outcome of *Plasmodium falciparum (Pf)* infections are not well understood. Although several immunological and genetic markers have been implicated, data on the impact of parasite diversity is lacking. This study aims to assess if *Pf* infections resulting in symptomatic or asymptomatic outcomes are genetically different. Children (n=997, aged 0.5-12 years) living in malaria endemic communities in the Greater Accra region, Ghana were recruited in a 50-week longitudinal cohort study. At baseline and monthly, thick and thin blood film and filter paper blots were made to determine asymptomatic parasite carriage. Similar samples and testing were done during symptomatic malaria episodes. A total 1800 SNP barcode and 42 resistance gene markers of the parasite genome was sequenced for each *Pf* positive sample using the Molecular Inversion Probe (MIP) technique on the Illumina NextSeq platform. Data was analysed using MIPtools bioinformatics pipelines and R. Overall, 974 children completed the 50-week follow up. Symptomatic *Pf* infection incidence was 17.9%. A total of 9,045 monthly parasitaemia surveillance samples were analysed by PCR showing 23.4% asymptomatic *Pf* infection. Symptomatic and asymptomatic *Pf* infection distribution was similar across the communities. Parasite diversity data may reveal crucial differences in parasites causing *Pf* infection. This may be useful in designing better malaria control tools.

1173

VEUPATHDB.ORG: EUKARYOTIC PATHOGEN, VECTOR AND HOST OMICS DATA MINING FOR EVERYONE

Susanne Warrenfeltz[1], Gloria I. Giraldo-Calderón[2], Omar S. Harb[3], Jessica C. Kissinger[1], Daniel Lawson[4], Mary Ann McDowell[2], David Roos[3], Samuel Rund[2], The VEuPathDB Team[1]

[1]*University of Georgia, Athens, GA, United States,* [2]*University of Notre Dame, Notre Dame, IN, United States,* [3]*University of Pennsylvania, Philadelphia, PA, United States,* [4]*Imperial College, London, United Kingdom*

VEuPathDB is one of two NIAID-funded Bioinformatics Resource Centers providing free, online, omics data mining resources that support arthropod vectors, eukaryotic pathogens, fungi, oomycetes and related organisms. The VEuPathDB family of resources includes VectorBase, PlasmoDB, TriTrypDB, FungiDB and more. These resources empower users to fully leverage omics data regardless of their computational skill level. Advanced search capabilities, data visualization and custom analysis tools are coupled to pre-analyzed omics data to facilitate hypothesis testing and the discovery of meaningful relationships from large volumes of data. Data types include genome sequence and annotation, transcriptomics, proteomics, epigenomics, metabolomics, population resequencing, variation data, clinical data, and host-pathogen interactions. VEuPathDB analyzes all data with documented standard bioinformatics workflows and in-house analyses generate data such as domain predictions and orthology profiles across all genomes. Data mining strategies range from simple browsing of records that compile all data for a gene or pathway; to data visualization in a genome browser; a unique search strategy system that queries the data and returns genes or features with shared biological characteristics; a private Galaxy workspace for analyzing user data in context with public data already integrated into EuPathDB; and the MapVEu tool for visualization, search, analysis and download of variation data. MapVEu integrates genomic, phenotypic and population data for traits such as insecticide resistance genotypes and phenotypes,

genetic variation with microsatellites, chromosomal inversions and SNPs, population abundance, pathogen infection status and blood meal identification. These free resources easily merge evidence from diverse data and across species to place the power of bioinformatics with every scientist. Our active user support offers an email help desk, social media, video tutorials, a webinars, and a worldwide program of workshops. Please stop by our exhibit hall booth, or email us at help@VEuPathDB.org for more information.

1174

DIFFERENTIAL GENE EXPRESSION ANALYSIS OF PBMCS FROM MALIAN CHILDREN REVEALS MOLECULAR MECHANISMS ASSOCIATED WITH VARYING SUSCEPTIBILITY TO CLINICAL MALARIA DISEASE

Gillian Mbambo[1], Ankit Dwivedi[1], Drissa Coulibaly[2], Abdoulaye K. Kone[2], Ando B. Guindo[2], Mahamadou A. Thera[2], Kirsten E. Lyke[3], Joana C. Silva[1]

[1]*Institute for Genome Sciences, 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 and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States*

Host gene expression impacts susceptibility to clinical malaria disease, but the immune cell subsets and genes that contribute to protection remain unidentified. To understand how gene expression patterns, influence clinical disease outcome, we collected and analyzed mRNA from peripheral blood mononuclear cells (PBMCs) collected from Malian children, aged 4-6 years, who differed in susceptibility to clinical malaria. Children were followed for 150 days throughout one malaria transmission season. Samples were collected at the beginning of the malaria transmission season (Day 0), peak malaria transmission season (Day 90), and the end of the malaria transmission season (Day 150). We characterized the functional, enzymatic, and cell sub-populations that are enriched at the three timepoints. To identify cell subsets and gene classes that play an important role in malaria disease outcome, we used differential gene expression analysis and gene set enrichment tools (IPA, GSEA and DAVID) on bulk mRNA sequence data. We observed that chemokine signaling pathways were enriched at the beginning of the transmission season in children who do not develop clinical malaria. Longitudinally, we observed enrichment of upstream regulators in co-stimulatory signaling pathways at the peak and the end of the malaria transmission season relative to the beginning of the transmission season. We will use deconvolution algorithms to determine which immune cell subpopulations are upregulated in disease pathogenesis. Our mRNA sequence analyses of immunologic cell subsets identified critical genes that regulate susceptibility to clinical malaria disease and defined prospective mechanisms of acquired protective immunity to malaria.

1175

POPULATION GENOMICS OF *PLASMODIUM VIVAX* FROM PANAMA USING SELECTIVE WHOLE GENOME AMPLIFICATION

Lucas Buyon[1], Angela Early[1], Marlon Nuñez[2], Sarah Volkman[1], Matthias Marti[3], Ana Maria Santamaria[2], Jose E. Calzada[2], Daniel E. Neafsey[1], Nicanor O. Rodriguez[2]

[1]*Harvard TH Chan School of Public Health, Boston, MA, United States,* [2]*Instituto Conmemorativo Gorgas de Estudios de la Salud, Panama City, Panama,* [3]*University of Glasgow, Glasgow, United Kingdom*

Malaria incidence in Panama has increased in recent years (~500 cases in 2010 to ~800 cases in 2017), despite elimination efforts, with the majority of cases caused by *Plasmodium vivax*. Notwithstanding, overall prevalence remains low (less than 1 case per 1000 persons). Genome sequencing of *P. vivax* parasites can provide insights into epidemiology and population structure. We used selective whole genome amplification to sequence 100 *P. vivax* samples from Panama collected between 2007 and 2019 to study the population structure and transmission dynamics of the parasite. Sixty samples yielded usable sequencing data. Four of the samples came from individuals with travel history, and imported cases resulting from enhanced levels of human migration are concern for malaria elimination prospects. We explored patterns of recent common ancestry among the samples and observed that a single parasite clone was dominant in the collection (47 out of 60), spanning the entire period of collection (2007-2019) and all regions of the country. We also found a second, smaller clonal lineage (n=4) collected during 2017-2019. To detect imported cases, we conducted principal components analysis and constructed a neighbor-joining tree using these samples and samples collected worldwide from a previous study. Three of the four samples with travel history clustered with samples collected from their suspected country of origin (confirming they were imported), while one did not. The small number of genetically unique Panamian *P. vivax* samples clustered with samples collected from Colombia, suggesting they represent the genetically similar ancestral *P. vivax* population in Panama or were recently imported from Colombia. The low clonal diversity we observe in Panama indicates that this parasite population is ripe for elimination. Additionally, while we confirmed that *P. vivax* is imported to Panama from diverse geographic locations, the lack of impact on the overall diversity of the parasite population suggests that onward transmission from such cases is limited and that imported cases will not pose a major barrier to successful elimination.

1176

SHORT-TERM LONGITUDINAL DYNAMICS OF MALARIA ANTIBODIES IN CHILDREN TREATED FOR *PLASMODIUM FALCIPARUM* INFECTION

Eric Rogier[1], Doug Nace[1], Pedro R. Dimbu[2], Brian Wakeman[1], Chris Drakeley[3], Kevin Tetteh[3], Mateusz Plucinski[1]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Angola National Malaria Control Program, Luanda, Angola,* [3]*London School of Hygiene & Tropical Medicine, London, United Kingdom*

Malaria infection in humans generates a robust adaptive immune response with leukocyte activation minutes after sporozoite inoculation by mosquito. Recent studies suggest malaria infection may be able to induce 'atypical' B cell responses and antibody secretion within a few days after antigen recognition. *Plasmodium falciparum* antigens have been identified to be primarily expressed during different stages of host infection, and antibody dynamics to many of these antigens are not well understood upon resolution of acute infection and convalescence. Here we tested longitudinal blood samples from 103 Angolan children followed for up to 42 days after initiation of malaria chemotherapy. Antibody levels for the five classes of immunoglobulins (Ig) and 4 subclasses of IgG were detected by multiplex bead assay in ability to bind 38 *P. falciparum* antigens which encompassed all 4 parasite life stages of human infection. At a 1:100 serum dilution, levels of IgD and IgE against these antigens were negligible, but all other Ig classes and IgG subclasses were consistently detected against all antigens. Antibody levels typically reached an apex within two weeks of parasite clearance, and consistent patterns by Ig class were observed for time to peak, magnitude of response, and modeled longevity in host. In contrast to IgM and IgG, IgA levels consistently showed a different pattern, rising in the first two weeks and hitting a nadir at 3-4 weeks post-treatment before rising again. After apex, antibodies levels of all Ig classes reliably displayed first-order exponential decay in removal from systemic circulation. Among the 38 antigens, variation was observed for the parameters of antibody acquisition, peak levels, and modeled loss, but these were not able to be generalized by parasite stage-specific expression patterns. These data show the human humoral response to *P. falciparum* is highly variable to different antigens, but that assessing Ig dynamics within the same person to multiple antigens can provide predictive capacity in determining a person's exposure history to this parasite.

1177

INFLUENCE OF CYTOKINE RATIO ON ANEMIA STATUS OF MALARIOUS CHILDREN IN SOUTH EASTERN NIGERIA

Chinyere Ihuarulam Okoro[1], Kingsley Achigbu[1], Oluchi Ijeoma Okoro[1], Francis Ihenetu[2], Ikechukwu Vincent Ejiogu[1], Lazarous Otukwude[1]

[1]*Federal Medical Center Owerri, Owerri, Nigeria,* [2]*Imo State University, Owerri, Owerri, Nigeria*

Interleukin 10 (IL-10) production appears to be important in the induction and maintenance of immunity to *P. falciparum* in naturally exposed populations. Down regulation of TNF-α production and consequent resistance to severe malaria, has been linked to the ability to produce the immuno-regulatory cytokine (IL-10), while a relative deficiency in immuno-regulatory cytokine (IL-10) and lower ratios of IL-10 to TNF-α has been recorded in patients with severe malaria. Children aged 1-72 months who presents with fever or history of fever in the last 24 hours at the selected Outpatient's Department of the Health facilities were enrolled after obtaining Ethical approval from the Research, Ethical Committee of Federal Medical Center Owerri Imo state. Blood samples were collected from respondents who consented for the diagnosis of malaria and anemia using outlined standard operating procedures (SOPs). Plasma/serum of all randomly selected children (both TEST and CONTROL) were freeze dried in aliquots of 100µl in cryoval tubes at –20°C until they were used for cytokine assays in accordance with the manufacturer's manual. The geometric mean parasite density of children positive by microscopy was 1764 parasites/µl of blood with a range of (12-220,000 000parsites/µl of blood). Anaemia ranged from mild to moderate, there was no severe malaria anaemia observed. A significant relationship was observed between anaemia and fever ($p < 0.001$), febrile children had higher percentage of mild and moderate anaemia than afebrile children (18.3% vs 15.0%) and (25.7% vs 15.0%).The geometric mean of IL10/TNF-α ratios of 2.8pg/ml, 2.1pg/ml and 1.7pg/ml were recorded for normal hemoglobin, mild and moderate anemia. The IL -10 and TNF concentrations increased respectively with advance in Anemia while the IL-10/TNF ratio decreased as Anemia advances. Increased IL-10/TNF-α ratio is associated with increased hemoglobin concentrations in acute, uncomplicated *P. falciparum* malaria ($p<0.001$).Thus, lower levels of IL-10 over TNF-alpha may contribute to development of malaria complications such as anaemia in addition to others factors

1178

IMMUNOGENICITY OF R21/MATRIX-M™ VACCINE IN MALARIA NAÏVE AND MALARIA ENDEMIC POPULATIONS

Duncan G. Bellamy[1], Mehreen Datoo[2], Rebecca Makinson[1], Mustapha Bitaye[1], Jeremy Aboagye[1], Amy Flaxman[1], Natama Magloire[3], Herman Sorgo[3], Ousman Traore[3], Meera Madhaven[2], Alfred B. Tiono[4], Jenny Reimer[5], Umesh Shaligram[6], Sodiomon B. Sirima[7], Halidou Tinto[7], Adrian V. Hill[1], Katie J. Ewer[1]

[1]*University of Oxford, Oxford, United Kingdom,* [2]*Centre for Clinical Vaccinology and Tropical Medicine, Oxford, United Kingdom,* [3]*IRSS-DRCO/CRUN, Nanoro, Burkina Faso,* [4]*Centre National de Recherche et Formation sur le Paludisme research unit, Banfora, Burkina Faso,* [5]*Novavax, Uppsala, Sweden,* [6]*Serum Institute of India, Pune, India,* [7]*Groupe de Recherche Action en Santé (GRAS), Ouagadougou, Burkina Faso*

Malaria is a leading cause of childhood mortality; there is need for an effective vaccine. R21 is a virus-like particle (VLP) based vaccine displaying the immunodominant NANP repeat region of the circumsporozoite protein (CSP) of *Plasmodium falciparum*. R21 is novel in that CSP epitopes cover the particle surface, whereas prominent malaria vaccine candidate, RTS,S, has a higher ratio of Hepatitis B surface antigen (HBsAg) to CSP. R21/Matrix-M adjuvant with a C-tag on the R21 manufactured in Oxford has been assessed in phase I/II trials to determine safety, immunogenicity and efficacy with different vaccine doses presenting with high anti-CSP titres. Recent manufacturing at the Serum Institute India have resulted in R21 manufactured without the C-tag. Comparable high levels of immunogencity is observed for VLPs made at both manufacturing sites. In the UK, regimen effects have been investigated by comparing 3 low doses administered one month apart (10,10,10µg) to a delayed (by 5 months) third dose. A significant 2-fold increase was observed in anti-IgG specific antibodies to the NANP repeat region of CSP in the delayed 3rd dose group. The magnitude and quality of immunogenicity to fractional third dose regimes (50,50,10µg & 10,10,2µg) is being investigated.A randomized phase IIb efficacy trial in 450 infants in Burkina Faso using low dose R21 (5ug) with either 25µg or 50µg of Matrix-M. 289/437 (66%) showed antigen specific IgG antibody reponses at D84 (Geometric mean 8256 EU, 7540-9040 95%CI), corresponding to the proportion of vaccinees receiving R21. This is a 5-fold increase in antibody response compared with UK adults (GM-1431 EU, 1092-1875 95%CI) and 16-fold increase compared with Burkinabe adults (477 EU, 206-1106 95%CI), indicating that R21 with adjuvanated with Matrix-M is more immunogenic in infants than adults in areas of significant malaria endemicity, which may in turn result in improved durability/efficacy. Additional studies of antibody isotype and subclass, antibody responses to the C-terminal region of the vaccine and T-Cell responses measure by IFNγ ELISPot will be undertaken to determine potential correlates of protection.

1180

PREVALENCE OF PVRBP1A, PVRBP2B, PVRII AND PVEBP, *PLASMODIUM VIVAX* PARASITE ANTIGENS AMONG PRIMARY SCHOOL CHILDREN IN A MALARIA ENDEMIC TRANSMISSION REGION IN SENEGAL

Aminata Colle Lo[1], Babacar Faye[1], Katie Patterson[2], Aminata Lam[1], Oumar Gaye[1], Christopher Drakeley[3]

[1]*University Cheikh Anta Diop of Dakar / Senegal, Dakar, Senegal,* [2]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [3]*london School of Hygiene and Tropical Medicine, London, United Kingdom*

Plasmodium vivax in one of the most prevalent of the global malaria cases. In the Sub-Saharan Africa, *P. vivax* was not present because of the absence of Duffy antigen receptor for chemokines in the surface of red blood cells (RBCs) in the majority of people. However, recently it was found some microscopic *P. vivax* parasites in some west African countries. This can be due to an alternative solution for erythrocyte invasion or people express Duffy antigen receptors. By molecular tool, data showed prevalence of *P. vivax* infection in children whom acquired earlier clinical immunity *P. vivax* compare to *P. falciparum* in endemic area. Studies showed that young children between 3 months to 3 years old living in endemic area, antibody to PvRBP1a & PvRBP2b were correlated to reduction of malaria clinical episode but also in asymptomatic Duffy-negative people in Kedougou. However, how immune system reacts to *Plasmodium vivax* parasite antigen? The emergence of *P. vivax* become a challenge for several countries moving for malaria elimination including Senegal. This work was undertaken in 6 primary schools between November 2018 and Mars 2019 and included a total of 430 children of age ranges from 5 to 11 years old. Participants were followed for four months and screened at monthly intervals for infection. Levels and prevalence of total IgG responses were quantified by LUMINEX with duplicate sera obtained from blood spots and diluted in 1:200 using standard procedure already described. We were able to test total IgG against four *P. vivax* parasites antigens which are PvRBP-1a, PvRBP-2b, PvRII and PvEBP. Among them, higher antibody response was observed against PvRBP1a and lower level of antibody against PvEBP. Antibody response to PvRBP-1a, PvRBP-2b, PvRII and PvEBP increased with age as showed in previous studies. Kendall correlation test was used to investigate association of antibody response to four antigens in four months follow-up. And between four *Plasmodium vivax* parasite antigens, PvEBP and PvRII were more correlated.

1181

CLINICAL MALARIA DRIVE T CELL EXHAUSTION MARKERS IN CHILDREN AND ADULTS

Oumar Attaher[1], Irfan Zaidi[2], Mamoudou B Samassekou[1], Djibrilla Issiaka[1], Sekouba Keita[1], Sibiri Sissoko[1], Tiangoua Traore[1], Kalifa Diarra[1], Bakary S Diarra[1], Adama Dembele[1], Gaoussou Santara[1], Moussa Traore[1], Almahamoudou Mahamar[1], Amadou Barry[1], Alassane Dicko[1], Patrick Duffy[2], Michal Fried[2]

[1]*Malaria Research & Training Center, Faculty of Medicine, Pharmacy & Dentistry, University of Sciences Techniques & Technologies of Bamako, Bamako, Mali,* [2]*Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy & Infectious Diseases, National Institutes of Health, Rockville, MD, United States*

Plasmodium falciparum infection is a devastating global health problem that has been exacerbated by emergence of drug resistant parasites. Acute malaria infections have been associated with an upregulation of T cell exhaustion markers such as programmed cell death-1 (PD-1) & lymphocyte activation gene -3 (LAG-3). In addition, PD-1 has been associated with decreased cytokine production & proliferation in T cells & this may delay parasite clearance or development of immunity. We previously showed that in children receiving seasonal malaria chemoprevention, reduction in malaria episodes led to a decrease in CD4+ PD1+ & CD4+PD1+LAG3+ T cells during the malaria transmission season. In the current study we compared the effect of malaria infections on the frequency of exhausted CD4 T cells (defined by expression of PD1+ & LAG3+) in adults & children. Both studies were conducted in an area of intense seasonal malaria transmission (July-December) in Ouelessebougou, Mali. After informed consent, participants were randomized to receive a single dose of Coartem at the start of the dry season. The levels of exhausted & regulatory T cells were measured every month for a period of 12-months using whole blood flow cytometry. During the malaria transmission season, percentage of CD4+ PD-1+ & CD4+PD1+LAG-3+ cells were significantly higher in malaria-infected children (n=73) compared to uninfected children (n=415) $p<0.001$. Further increase in percentage of CD4+ cells expressing PD1 & LAG3 was observed in children with clinical malaria (n=57) compared to asymptomatic children (n=16), $p=0.009$ & $p=0.003$. In adults CD4+PD1+ levels were similar between infected (n=103) & uninfected (n=452) individuals, $p=0.576$ while CD4+PD1+LAG-3+ were significantly higher in malaria-infected compared to uninfected adults $p<0.001$. These results suggest that malaria infection has a larger impact on immune exhaustion in children than in adults and clinical malaria further increased immune dysfunction.

1182

WILD-DERIVED MICE AS A MODEL FOR ASYMPTOMATIC MALARIA

Anne Majgaard Jensen, Douglas H. Cornwall, Adesola C. Olatunde, Mellina T. Srey, Franklin Maloba, Birk K. Evavold, Brian D. Evavold, Tracey J. Lamb

University of Utah, Salt Lake City, UT, United States

Individuals with asymptomatic malaria are a potential reservoir of disease transmission and pose a significant threat to the control of malaria worldwide. Asymptomatic malaria is usually defined as malaria without overt symptoms although mild anemia is often present and individuals tend to be more susceptible to bacterial co-infections. Asymptomatic malaria can occur at all ages. In older individuals asymptomatic malaria is thought to be associated with the development of the adaptive immune response after cumulative exposure to *Plasmodium spp*. However, in young children under the age of 2 asymptomatic malaria occurs prior to the development of robust adaptive immunity. In this age group we hypothesize that differences in the immune response controlled by genetic variation of the host determines the outcome of disease. There is currently no mouse model for asymptomatic malaria. Here we utilize a specific pathogen free (SPF) wild-derived *Mus musculus domesticus* mouse strain with a similar amount of genetic variation to that of humans to demonstrate genetic control of asymptomatic malaria. When infected with *Plasmodium yoelii* XNL wild-derived mice display a large variation of anemia ranging from asymptomatic to severe. To explore the immunological correlates of anemia severity, we determine splenic responses at the end of the infection period (day 19 post-infection) and saw that innate immune responses were significantly correlated with the severity of anemia. In general severity of anemia in symptomatic wild-derived mice were associated with a higher proinflammatory response compared to the asymptomatic wild mice. In particular TNF-α, as expected, appeared to be involved in inducing anemia as might be expected from published studies. These findings suggest that using the wild-derived mice to model genetic diversity within human populations may enable us to pinpoint the immunological nature of the asymptomatic malaria.

1183

INVESTIGATING IMMUNE SIGNATURES PREDICTIVE OF INCIDENT *PLASMODIUM FALCIPARUM* INFECTIONS IN MALIAN CHILDREN

Jyoti Bhardwaj[1], Leetah C. Senkpeil[2], Aditi S. Upadhye[1], Aissata Ongoiba[3], Joaquin Cardozo[4], Aarti Jain[5], Safiatou Doumbo[3], Kassoum Kayentao[3], Hongyu Gao[6], Hans Ackerman[7], Xiaoling Xuei[6], Phillip L. Felgner[5], Yunlong Liu[6], Boubacar Traoré[3], Peter D. Crompton[8], Tuan M. Tran[1]

[1]*Division of Infectious Diseases, Department of Medicine, Indiana University School of Medicine, Indianapolis, IN, United States,* [2]*Indiana Bio-Medical Gateway Program, Indiana University School of Medicine, Indianapolis, IN, United States,* [3]*Mali International Center of Excellence in Research, University of Sciences, Technique and Technology of Bamako, Bamako, Mali,* [4]*Oberlin College, Oberlin, OH, United States,* [5]*Division of Infectious Diseases, Department of Medicine, University of California, Irvine, CA, United States,* [6]*Department of Medical & Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, United States,* [7]*Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, Rockville, MD, United States,* [8]*Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States*

Clinical immunity to malaria can be acquired naturally in highly malaria-endemic settings. In contrast, natural immunity that prevents *Plasmodium* infection is rarely achieved even after years of repeated malaria exposure. In a prospective cohort study conducted in Mali, we identified a subset of young children who never demonstrated *P. falciparum* (*Pf*) parasitemia (n=12) during the first malaria season despite intensive surveillance over 7 months consisting of PCR-based screening of blood collected every 2 weeks and during sick visits. To determine host immune signatures predictive of this aparasitemic phenotype, we performed *Pf*-specific IgM and IgG profiling by protein array, whole-blood transcriptome profiling by RNA-Seq, and multiplex cytokine analysis using blood collected at the beginning of the study. Age- and sex-matched children who became parasitemic during the same season were used as controls. Plasma antibody profiling against 250 *Pf* antigens revealed boosting of *Pf*-specific IgG antibodies during the malaria season in nine children, showing evidence of parasite exposure and suggestive of naturally occurring sterile protection. *Pf*-specific IgM/IgG profiles at baseline did not differ significantly between the two classes. Transcriptomic analysis showed no significant differential expression at the gene level but revealed significant enrichment of several blood transcription modules. Relative to parasitemic children, aparasitemic children demonstrated upregulation of modules related to neutrophils, B cells, and signal transduction modules and downregulation of modules related to PLK signaling, T cells, NK cells, cell cycle regulation, E2F targets, and the interferon-γ response. Pro-inflammatory cytokines including TNF-α and IL-8 were increased in aparasitemic children relative to parasitemic children. Taken together, this data suggests that prospective parasitemia outcomes can be defined by baseline transcriptional and plasma cytokine signatures. This study provides insights into protective immunity against *Pf* infection that can help inform the design of next-generation malaria vaccines.

1184

ELEVATED ADMISSION SERUM URIC ACID LEVELS ARE ASSOCIATED WITH MORTALITY IN UGANDAN CHILDREN WITH SEVERE MALARIA

Deepali Balasubramani[1], Katrina Co[1], Dibyadyuti Datta[1], Ruth Namazzi[2], Andrea L. Conroy[1], Robert O. Opoka[2], Chandy C. John[1]

[1]*Ryan White Center for Pediatric Infectious Disease and Global Health, Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN, United States,* [2]*Department of Paediatrics and Child Health, Makerere University, Kampala, Uganda, Kampala, Uganda*

In the pathology of inflammatory diseases like severe *Plasmodium falciparum* malaria, elevated uric acid (UA) levels released from dying cells can act as a 'danger signal' triggering immune activation. Highly elevated UA levels resulting in the formation of UA crystals have been associated with an increased risk of mortality in sepsis, cardiovascular disease, and kidney disease. However, its role in mortality associated with severe malaria is unclear. In this study, we investigated the role of UA in pathogenic outcomes associated with severe malaria. We enrolled 720 Ugandan children ages 6 months-4 years into one of five severe malaria categories - cerebral malaria (CM), respiratory distress (RD), malaria with complicated seizures (M/S), severe malarial anemia (SMA), and prostration (PRS). Healthy community controls (CC) were enrolled from the same neighborhoods of children with severe malaria. Serum UA levels were quantified using a modified Fossati method to measure hydrogen peroxide produced by the uricase reaction. Admission UA concentrations in children with CM (median [IQR], mg/dL; 7.4 [4.8-11.7]), RD (7.8 [5.6-10.8]), M/S (4.5 [3.7-5.4]), SMA (5.8 [4.1-8]), and PRS (4.4 [3.3-5.6]) were all significantly elevated compared to CC (3.7 [3-4.3]; all $p<0.05$). UA concentrations in severe malaria groups with the highest mortality (CM and RD) (7.7 mg/dl [5.2-11.1]) were significantly higher compared to the groups with low or no mortality (M/S, SMA, and PRS) (4.8 mg/dl [3.8-6.5], $p<0.001$). UA concentrations in children with CM or RD were elevated beyond the threshold of precipitation that leads to UA crystal formation (>7 mg/dl), and among children with CM or RD, those who died had significantly elevated UA levels (11.1 mg/dl [7.2-13.2]) compared to those who survived (7.3 mg/dl [4.9-10.3], $p<0.001$). Our findings show that UA levels in severe malaria increase with increasing disease severity and are associated with mortality. Future studies will determine associations between elevated UA levels and other factors related to disease severity in severe malaria.

1185

ANTIBODY RESPONSES TO REPETITIVE CONTROLLED HUMAN MALARIA INFECTIONS IN MALARIA-NAÏVE ADULTS USING NF54 STRAIN *PLASMODIUM FALCIPARUM*

Patricia Ferrer[1], Allison N. Bucsan[1], Tao Li[2], Abraham Eappen[3], Sumana Chakravarty[4], Anusha Gunesekera[4], Anita Manoj[4], Faith Pa'ahana-Brown[5], Effie Nomicos[6], B. Kim Lee Sim[2], Stephen L. Hoffman[2], Andrea A. Berry[5], Kirsten E. Lyke[5], Kim C. Williamson[1]

[1]*Uniformed Services University of the Health Sciences, Bethesda, MD, United States,* [2]*Sanaria, Inc. and Protein Potential, Inc., Rockville, MD, United States,* [3]*Protein Potential, Inc., Rockville, MD, United States,* [4]*Sanaria, Inc., Rockville, MD, United States,* [5]*University of Maryland Center for Vaccine Development and Global Health, Baltimore, MD, United States,* [6]*Parasitology and International Programs Branch, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States*

Currently, sequential natural malaria infections remain the primary means of inducing protective immunity, but the targets and mechanisms are not well understood. Using repetitive controlled human malaria infection (CHMI) with *Plasmodium falciparum* (Pf) strain NF54, we monitored parasitemia and antibody responses in volunteers through four sequential infections. After exposure to a mock CHMI with bites of 5 uninfected *Anopheles stephensi*, the volunteers were challenged with 5 PfNF54-infected mosquitoes 2, 9, 14 and 23 months later. Antimalarials were administered when parasitemia was detected by thick blood smear. Plasma was collected on days 1, 6, 8, treatment day, and 7 days post-treatment. The pre-patent period increased from 11.3±1.2 days in CHMI1 to 13.8±2.5 days in CHMI4; two volunteers had a delay to patency until days 16 and 17. As a first screen, plasma was tested for anti-PfCSP and PfGLURP-R2 repeat Ig titers. Ig responses varied, but by 7 days post-treatment following CHMI3 all 8 volunteers had IgG against PfGLURP-R2 and only one did not have anti-PfCSP IgG. Notably, the two volunteers with delayed pre-patent periods after CHMI4 had anti-PfCSP IgG on D1 of CHMI4, suggesting sustained PfCSP IgG responses may be associated with partial pre-erythrocytic immunity. Except for these two volunteers, after each CHMI the PfCSP and PfGLURP-R2 IgG responses at 7 days post treatment decreased to baseline by D1 of the next CHMI and did not increase again until treatment day for two volunteers and 7 days post treatment for three. The two volunteers with increased anti-PfCSP IgG on treatment day of CHMI3 also had a 4 day delay to patency. Although transient, the anti-PfCSP and PfGLURP-R2 IgG levels observed 7 days after treatment significantly increased after each CHMI in all but two volunteers. These results demonstrate that most volunteers produced antibodies against Pf, but that impact on the parasites was associated with the transition to maintaining IgG levels. Further evaluation, including subsequent challenges, of volunteers who have and have not made this transition will be key to understanding the development of protection.

1186

COMPARATIVE TRANSCRIPTOMIC ANALYSIS OF THE PRIMATE IMMUNE RESPONSE TO MALARIA AND MALARIA-LIKE PARASITES

Amber Eliza Trujillo[1], Christina M. Bergey[2]

[1]*Department of Anthropology, New York University, New York, NY, United States,* [2]*Department of Genetics, Rutgers University, Piscataway, NJ, United States*

A comparative analysis of host response to malaria (*Plasmodium* spp.) and malaria-like parasites (*e.g., Hepatocystis*) has the potential to elucidate the underlying genetic architecture of disease response in humans. However, few studies of host-pathogen dynamics and the genetic architecture of malaria resistance and susceptibility have been undertaken in wild populations of non-human primates. Furthermore, most research has focused on a single or few loci; however, variation at these loci does not explain all of the variation in the malaria resistance/susceptibility phenotype. Using previously published datasets, we analyzed blood transcriptome (RNAseq) data from Ugandan red colobus monkeys (*Piliocolobus tephrosceles*) affected by *Hepatocystis*. We inferred malaria parasitemia by calculating the ratio of sequencing reads with closest matches to the host and pathogen reference genomes within each monkey. We identified genes with expression that was significantly positively correlated with parasitemia, and we tested for the enrichment of functional pathways in these genes. We found that the genes with expression most strongly positively correlated with parasitemia included *LSM14A* and *SLC16A1*. The Gene Ontology biological process with the strongest evidence of enrichment among colobus monkey genes with expression significantly positively correlated to parasitemia was erythrocyte differentiation (adj $p<0.05$). Similarly, enriched phenotypes, inferred via orthology between human-colobus genes, included two disorders involving red blood cells: poikilocytosis, or the production of abnormally shaped erythrocytes (adj $p<0.05$) and reticulocytosis, or the production of immature erythrocytes (adj $p<0.05$), which is disrupted during malaria anemia in humans. This study illustrates the utility of comparative research into pathogen response immunogenetics using natural transmission systems. Ultimately, investigating immune response to malaria across non-human primates may identify potential points of intervention that could help alleviate the huge burden that malaria continues to exert on human populations.

1187

MESSENGER RNA EXPRESSING PFCSP INDUCES FUNCTIONAL, PROTECTIVE IMMUNE RESPONSES AGAINST MALARIA IN MICE

Katherine Mallory[1], Neeraja Punde[1], Justin Taylor[1], Michael Sibilo[1], Ishita Waghela[1], Cosette Schneider[1], Xiaoyan Zou[2], Sri Hadiwidjojo[2], Leah Perazzo[1], Tatyana Savransky[1], Paulo Lin[3], Chris Janse[4], Sheetij Dutta[1], Ying Tam[3], Evelina Angov[1]

[1]*Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [2]*Naval Medical Research Center, Silver Spring, MD, United States,* [3]*Acuitas Therapeutics, Vancouver, BC, Canada,* [4]*Leiden University Medical Center, Leiden, Netherlands*

Malaria caused 228,000,000 cases and 405,000 deaths worldwide in 2018 alone (World Malaria Report 2019). Strategies for infection control of this global pathogen include a variety of approaches, such as insecticides, anti-malarial drugs, biologics (monoclonal antibodies) and vaccines. Needless to say, the ever evolving emergence of vector insecticide-resistance and parasite drug-resistance demands the development of safe, durable and broadly effective malaria vaccines. Currently, many malaria vaccine candidates in development are based on the dominant coat antigen of the invasive stage of the most prevalent and lethal malaria species, the circumsporozoite protein of *Plasmodium falciparum* (PfCSP). This antigen is the foundation of the most clinically advanced vaccine product to date. Despite decades of extensive research, dozens of clinical trials, and a four immunization strategy, protection levels of this vaccine in the field hover around 30-40% with quickly waning responses, highlighting the need for a more effective prophylactic option (i.e. RTS,S). Recent advances in mRNA technology for stable, targeted antigen delivery make this platform a highly attractive alternative to conventional vaccine approaches, such as DNA, recombinant protein, and viral-vectored platforms. In pursuing an immunogenic, protective vaccine against malaria, a *pfcsp* mRNA construct was synthesized by a commercial vendor. This product was tested in mice with a range of promising LNPs, mRNA doses, and immunization regimens. Initial studies indicate that this construct is capable of eliciting potent, functional humoral responses, as well as staggering cellular responses. Following optimization of a variety of factors to maximize immunogenicity, *pfcsp* mRNA was tested in a murine protection model using transgenic rodent parasites expressing the 3D7 strain PfCSP. In this preliminary study, a high dose prime and boost regimen of *pfcsp* mRNA/LNP induced 40% sterile protection in Balb/c mice. While these findings are encouraging, further work is needed to assess the potential of mRNA/LNPs as an effective vaccination approach against malaria infection.

1188

ANTIBODY SIGNATURES ASSOCIATED WITH DIFFERENT MALARIA TRANSMISSION INTENSITIES IN CHILDREN DURING ACUTE *PLASMODIUM FALCIPARUM* INFECTION AND CONVALESCENCE

Nancy Kemuma Nyakoe[1], Collins M. Morang'a[1], Tate Oulton[2], Kevin Tetteh[2], Jean Langhorne[3], Gordon Awandare[1], Yaw Bediako[1]

[1]*West African Centre for Cell Biology of infectious Pathogens (WACCBIP), Accra, Ghana,* [2]*London School of Hygiene and Tropical medicine, London, United Kingdom,* [3]*The Francis Crick Institute, London, United Kingdom*

Antibodies play a crucial role in naturally acquired immunity to malaria and can serve as biosignatures of protective immunity. The varied exposure to *Plasmodium falciparum* as observed in malaria-endemic areas causes distinct accumulation of specific antibody responses to various antigens. However, the distinct antigen repertoire that is responsible for protective antibody response is still not clearly defined. Traditional approaches to understanding the host's humoral immune response are unable to provide an integrated understanding of the antibody repertoire generated in response to infection. This study used machine learning methods to identify predictive antibody signatures that can model association with immune status and infection. Antibody responses in children aged 5-14 years were profiled during acute *P. Falciparum* infection (D0) and convalescent (D7 and D21) with protein microarray. This was a longitudinal hospital-based study in two regions in Ghana with distinct malaria transmission intensities: Accra (low transmission) and Kintampo (high transmission). Hierarchical clustering revealed antibody responses to certain malaria antigens had distinct IgG profiles in the two epidemiological areas. Of the 190 antigens tested, 48 antigens were significantly immunoreactive in low transmission areas and 30 antigens in the high transmission area. Antibody levels to most of the antigens seemed to persist even during the convalescent period. Multivariable linear regression models predicted age and transmission intensity as factors that highly affect antibody reactivity to most antigen in our panel. Machine learning modeling further predicted 18 signature antibody responses that can distinguish children from different transmission areas. This study identified an antibody repertoire that distinguishes individuals with different immune status, the composition of this signature can be used to predict immunity. The identified antigens could be useful as possible key targets of protective immunity that provide clues for potential vaccine candidates.

1189

PRIORITIZATION OF INSECTICIDE-TREATED NET INTERVENTIONS IN HAITI, USING A GEOGRAPHICALLY CONNECTED MATHEMATICAL MODEL FOR MALARIA

Clara Champagne[1], Marc Aurèle Telfort[2], Darlie Antoine[2], Emmanuel Forlack Allo[3], Phoebe Meyer[4], Sharif Egal[4], Justin Lana[4], Paola Blendl[4], Perez Luis[4], Nick Ruktanonchai[5], Maximilian Gerhards[1], Ewan Cameron[6], Katherine Battle[6], Emilie Pothin[1]

[1]*SwissTPH/University of Basel, Basel, Switzerland,* [2]*Programme National de Contrôle de la Malaria, Ministère de la Santé Publique et de la Population, Port-au-Prince, Haiti,* [3]*Haiti Country Office, Pan American Health Organization/World Health Organization, Port-au-Prince, Haiti,* [4]*Clinton Health Access Initiative, New York, NY, United States,* [5]*School of Geography & Environmental Science, University of Southampton, Southampton, United Kingdom,* [6]*Malaria Atlas Project, Oxford Big Data Institute, Li Ka Shing Centre for Health Information and Discovery, University of Oxford, Oxford, United Kingdom*

As Haiti approaches malaria elimination, malaria risk will undoubtedly become more concentrated to smaller geographic areas. In response, interventions must be carried out in a targeted fashion to maximize effect given finite resources. Typically, interventions are targeted using case counts and incidence as proxies for malaria risk. However, in order to maximize the effect of interventions and to maintain gains toward elimination, population and parasite movement (i.e. local importation patterns) must also be considered. For this purpose, we use a metapopulation model to represent malaria transmission in Haiti and to identify the communes, in which Long Lasting Insecticide-Treated Nets (LLIN) distributions would have most impact at the national scale. The model includes connectivity across the Haitian communes quantified using call detail records data, as well as past interventions such as case management and LLIN distributions. The model is fitted to the historical trend of reported malaria case counts between 2014 and 2018 using MCMC methods. Several versions of the model are compared to evaluate the sensitivity to the input data. The fitted model enables the simulation of the impact of future intervention scenarios and was used to identify the communes, in which LLIN distributions would have most impact at the national scale, thus providing a prioritised list of communes for interventions. As this model combines the effects of connectivity, past interventions and local trends in transmission intensity, it can provide a useful additional piece of information to help in the identification of intervention strata. These results were used to inform the selection of the communes for the 2020 bednet distribution campaign, in complement to the stratification approach used by PAHO. In particular, our model identified four communes for targeting that would have otherwise been excluded from receiving LLIN on the basis of case-counts/incidence alone. Finally, this calibrated model is a useful additional tool to support strategic decisions including the 2021-2023 Global Fund application.

1190

INVESTIGATION OF PARASITE-HOST DYNAMICS IN ANTIMALARIAL DRUG DEVELOPMENT REVEALS A DISCONNECT IN EXPERIMENTAL ENDPOINTS

Lydia Burgert[1], Sophie Zaloumis[2], Saber Dini[2], Louise Marquart[3], Pengxing Cao[4], Mohammed Cherkaoui[5], Nathalie Gobeau[5], Matthias Rottmann[1], Sergio Wittlin[1], James McCarthy[3], Julie A. Simpson[2], Jörg J. Möhrle[5], Melissa A. Penny[1]

[1]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [2]*Centre for Epidemiology and Biostatistics, Melbourne, Australia,* [3]*QIMR Berghofer Medical Research Institute, Brisbane, Australia,* [4]*School of Mathematics and Statistics, Melbourne, Australia,* [5]*Medicines for Malaria Venture, Geneva, Switzerland*

The antimalarial drug development pipeline assesses drug efficacy in murine malarial infection in *P. berghei* - NMRI mice and *P. falciparum* - NOD $^{scidIL\text{-}2R'\ c\text{-}/\text{-}}$ mice (SCID-mice) experiments before proceeding to human volunteer infection studies with *P. falciparum*. We investigated the appropriateness of translation of efficacy between experimental systems by mathematically modelling parasite growth and drug responses in murine and human malaria infections. Models were calibrated to extensive parasite growth and treatment data in the respective experimental systems for several compounds from the Medicines for Malaria Venture portfolio. General comparison of experimental set-up and parasite-host combinations between preclinical and clinical testing systems show differing scales and progression of infection, in terms of inocula, parasite growth and parasitemia reached, which implies drug effects are compared on different parasite loads in human and murine experiments. We compared the influence of parasite-host dynamics throughout the development pathway on parasite clearance induced by treatment since they are routinely used as an indicator of drug efficacy. Sensitivity analysis revealed the dependence of parasite clearance on experimental background e.g. human RBC injections in SCID-mice and/or adaptive host dynamics. In SCID mice, erythrocyte clearance plays a key role, whereas for *P. berghei* - NMRI infection and *P. falciparum*-human infection parameters of drug action and their interactions are more influential. Analysis of endpoints linked to cure and recrudescence was hindered by insufficient data on cure rates in murine and human malaria infection and missing mechanistic insights for a conclusive mechanistic translation. Overall, a disconnect in endpoints between the preclinical and clinical testing stages was discovered through mechanistic modelling, leading to an aggravation of translatability of mechanistic insights into parasite-host-drug dynamics between the different clinical stages moving towards clinical field studies.

1191

ESTIMATING HAPLOTYPE FREQUENCIES AND PREVALENCE ALONGSIDE MULTIPLICITY OF INFECTION FROM MALARIA SNPS DATA

Henri Christian Junior Tsoungui Obama, Kristan Alexander Schneider

Hochschule Mittweida, Mittweida, Germany

The introduction of SNP barcodes in *P. vivax* and *P. falciparum* facilitated standardized genetic analysis of malaria, being revealing about the transmission patterns on temporal and spatial scales, based on haplotype characterization. Moreover, estimating frequencies and prevalence of drug-resistance-associated haplotypes, characterized by several SNPs at resistance-associated loci, as the K-13propeller locus associated with artemisinin resistance, is part of routine monitoring. Unfortunately, the estimation of haplotypes is hampered by ambiguous genetic information resulting from the presence of multiple genetically different haplotypes within a single infection, known as multiplicity of infection (MOI). Consequently, haplotype-based analysis often relies on ad hoc estimations and deflated data, resulting from the removal of samples with ambiguous genetic information. This leads to less confident and substantially biased estimates. Methods based on statistical models often take Bayesian approaches yielding in the strict sense posterior distributions rather than desired point estimates. Typically, the properties of these methods are insufficiently understood. Here we introduce a statistical framework to obtain maximum-likelihood (ML) estimates of haplotype frequencies/prevalences alongside MOI from malaria SNP data. Unfortunately, no closed solution maximizing the likelihood function exists and the estimates need to be derived numerically. Because numerical maximization in high dimensional space is notoriously difficult, we adapt the EM algorithm, to maximize the likelihood function, which offers an efficient and numerically stable solution. The ML estimate has the typical desirable asymptotic properties. We conduct a systematic numerical study, to explore the statistical properties of the method for realistic sample sizes and provide an easy to use implementation. The method performs well for reasonable sample sizes and number of loci. Importantly the model has the potential to be generalized to arbitrary genetic architectures. Finally, we apply the method to a data set collected in Yaounde, Cameroon.

1192

THE SPREAD OF DRUG RESISTANCE IN *PLASMODIUM VIVAX VS. P. FALCIPARUM* MALARIA: THE EFFECT OF HYPNOZOITES

Sulyman Iyanda[1], Nessma Yousif[1], Kristan A. Schneider[2]

[1]*African Institute for Mathematical Sciences (AIMS), Limbe, Cameroon,* [2]*University of applied sciences,, Mittweida, Germany*

Despite the efforts to control and eradicate malaria worldwide, it remains one of the major challenges to global development. The use of anti-malarial drugs still is the keystone in malaria control and prevention. However, the spread of drug resistance in *P. falciparum* is a serious threat. While many drugs became ineffective to treat *P. falciparum* malaria, drug-resistance is uncommon in *P. vivax* malaria. It has been argued that differences in the life-histories of the two species, determining fitness-components lead to a more effective mechanism selecting for resistance in *P. falciparum*. These include in particular the onset of gametocytogenesis and the longevity of gametocytes. The presence of hypnozoites in *P. vivax* is a further life-history difference that has not been taken into account yet. Here we introduce a population-genetic model for the evolutionary dynamics of anti-malaria drug resistance. We explain how the presence of dormant hypnozoites delays the evolutionary process underlying drug resistance in *P. vivax* compared with *P. falciparum*. Importantly, the presence of hypnozoites (and hence relapses) does not affect fitness driving drug resistance, but only delays the evolutionary dynamics. The model per se is applicable to all species of malaria.

1193

MITIGATING THE IMPACT OF COVID-19 ON MALARIA BURDEN IN BURKINA FASO WITH CHEMOPREVENTION STRATEGIES: A MODELING STUDY

Aadrita Nandi[1], Monique Ambrose[2], Ifeoma D. Ozodiegwu[1], Caitlin Bever[2], Jailine Gerardin[1]

[1]*Northwestern University, Chicago, IL, United States,* [2]*Institute for Disease Modeling, Bellevue, WA, United States*

Malaria is a leading cause of morbidity and mortality in Burkina Faso, with over 10 million cases and over 12,000 deaths in 2018. With the COVID-19 pandemic potentially resulting in a large number of cases in Burkina Faso, measures such as social distancing interventions initiated to control the spread of the virus may affect malaria control interventions, and facilities overburdened with COVID-19 patients may be less able to treat malaria. In particular, sick and vulnerable groups, such as children under the age of five, may have limited access to testing and case management at health care facilities and administration of seasonal malaria chemoprevention (SMC) may be impacted. Using a stochastic, individual-based simulation framework, we estimated the impact of reduced case management on malaria burden and considered possible mitigations such as expanded SMC and mass drug administration (MDA) to counter the impact of loss of access to treatment due to COVID-19. We predict that a 75% decrease in case management will increase malaria mortality in children under the age of five by 65-115% depending on the case fatality rate of untreated

severe malaria. To mitigate the adverse effects of the pandemic, we consider the expansion of administration of SMC to older children as well as MDA and evaluate how much these alternate interventions can reduce the excess mortality. Our results suggest that expanding SMC to children under 10 or under 15 years of age can partially mitigate the impact of reduced case management. If case management is reduced by 75% and SMC is expanded to children under 15, mortality in children under five is still increased by 55-103% relative to a baseline with no COVID-19 disruptions. Expansion of SMC to all ages further reduced but did not eliminate excess mortality, and a single round of MDA was less effective than expanding SMC. To maintain current mortality rate despite a decline in case management, other interventions will be required along with expansion of SMC.

1195

MAPPING HUMAN DWELLINGS WITH REMOTE SENSING AND MACHINE LEARNING METHOD IN RURAL ETHIOPIA

Ming-Chieh Lee, Hao Tang, Xiaohui Xie, Guiyun Yan

University of California, Irvine, Irvine, CA, United States

House location represents one of the most important variables in the assessment of exposure to vector-borne pathogens. Accurate information on house location can improve modeling disease transmission risk and help planning interventions. However, obtaining accurate house location in vast rural areas of Africa where there was no prior detailed mapping effort is labor-intensive, expensive, and time-consuming. High-resolution remote sensing images are useful, but the traditional method to manually identify house structure needs skilled technicians and also prone to errors due to morphological resemblance of ground objects to human dwellings. We conducted a case study by combining remote sensing and machine learning methods to detect human dwelling in a rural area in southwest Ethiopia. Using high resolution, pan-sharpened Pléiades satellite images, and labeled data from the ground survey, a deep convolutional neural network for object detection following by the Faster-RCNN method was used to generate low-level features and high-level features. Bounded box regression and classification were conducted. The model was trained on a 10 x 10 km area of images with 1471 labeled houses and tested on another three 10 x 10 km area of satellite images in the nearby area. The model yielded an overall accuracy of 75% in the area and 88.2% accuracy on the corrugated iron sheet roof type houses. We are currently testing several new machine learning algorithms to improve model prediction accuracy. A combination of remote sensing and machine learning methods is valuable to detect human dwellings, estimate human density, and the population under risk in rural Africa.

1196

A DETAILED MODEL OF *PLASMODIUM FALCIPARUM* RECOMBINATION RUNS MODULARLY ON TRANSMISSION TREES TO PROVIDE NEW INSIGHTS ON POPULATION GENETIC DYNAMICS

Albert Lee, Jonathan Russell, Daniel Bridenbecker, Jessica Ribado, Caitlin Bever, Edward Wenger, Joshua L. Proctor

Institute for Disease Modeling, Bellevue, WA, United States

We present a modular framework (GenEpi) for interfacing genetic models of malaria parasites with transmission trees. GenEpi has the flexibility to run different models and realizations of parasite genetics on the same tree, which we leverage to study the connections between genetic features, epidemiological dynamics, and underlying assumptions about population diversity. The model incorporates the effects of gametocyte densities, oocyst development, and hepatocyte formation in order to realistically simulate the statistical properties of recombination and selection. We examine the sensitivities of genetic features to different evolutionary models and initial conditions of diversity, and pressure test correlations observed in genomic data. GenEpi can link with any external model that exports standard transmission tree formats, as demonstrated by a two-step pipeline that layers GenEpi onto trees generated by EMOD, a stochastic agent-based model of malaria transmission. This not only increases the efficiency of model optimization and calibration, but also better characterizes the interaction between the genetics and epidemiology. While EMOD gives a complete picture of transmission dynamics with agent-based representations of individuals, vectors, and parasites that are all calibrated to region-specific data, GenEpi offers a detailed view of parasite genetics, capturing the dynamics of cotransmission up to arbitrarily high complexities and calibrating initial conditions of population diversity to longitudinal genomic data. We have released GenEpi as an open-source Python package available on Github. We present analyses using GenEpi to relate uncertainties in inferred estimates of prevalence and incidence to the variance in genetic diversity, as well as any correlations with other epidemiological meta-data. We further show the effect that force of infection may have on this signal in different transmission settings, with focus on scenarios of importation vs local circulation. Finally, we examine the sensitivities of these signals to different modes of sampling and types of sequencing technologies.

1197

UNDERSTANDING THE DETERMINANTS OF THE SPREAD OF ANTIMALARIAL DRUG RESISTANCE USING AN INDIVIDUAL-BASED MODEL

Thiery Masserey, Tamsin Lee, Monica Golumbeanu, Andrew James Shattock, Melissa Penny

Swiss Tropical and Public Health Institut, Basel, Switzerland

Artemisinin Combined Therapies (ACTs) are the most up to date treatment for malaria. However, resistance to artemisinin, and its partner drugs, represent a pressing threat to malaria elimination. The drivers of the spread of drug resistant genotypes range from the access to health care, to the biology of the resistant organism, to the pharmacological properties of the drug. To mitigate the spread of drug resistant genotypes, one must first quantify which drivers of the spread are key, and whether this varies under different settings. To address this problem systematically, we use an established mathematical model, Open Malaria. This is a stochastic individual-based model that captures a wide range of important aspects of malaria epidemiology, including the pharmacokinetic/pharmacodynamics and various settings. We determined the key drivers of the spread of drug resistance for two different drug profiles: short-acting and long-acting. Short-acting drugs have a short half-life with a high killing rate (like artemisinin), and long-acting drugs have a long half-life with a low killing rate (like partner drugs to artemisinin). We considered regions with seasonality variation and regions without. Via a global sensitivity analysis, we showed that for both short-acting and long-acting drugs, the treatment rate, the level of drug sensitivity of the resistant genotype, the fitness cost, and the transmission intensity influence the spread of resistance. For long-acting drugs, when the drug half-life was long, the spread of drug-resistant genotype was fast. However, for short-acting drugs, a longer period of drug exposure seems to reduce the spread of resistant genotypes. Our analysis suggests that seasonality reduces the spread of the resistant genotype because of an increased influence of the fitness cost, and a reduced influence of the half-life during the low transmission period. Our results highlight that drug access and properties have an important role in the spread of resistance. This work focused on the use of drugs as monotherapy, which provides the necessary foundation for a similar analysis when the drugs are used in combination.

1198

A SPATIAL EPIDEMIOLOGICAL-GENETIC MODEL TO SUPPORT COUNTRY PROGRAM DECISION-MAKING IN MALARIA CONTROL AND ELIMINATION STRATEGY

Jonathan R. Russell, Albert Lee, Daniel Bridenbecker, Jessica Ribado, Katherine Battle, Edward A. Wenger, Joshua L. Proctor, Caitlin A. Bever

Institute for Disease Modeling, Bellevue, WA, United States

Genomic surveillance has the potential to complement existing surveillance tools to inform malaria control and elimination strategy. Spatial epidemiological-genetic models have the capacity to integrate genomic data with other traditional parasitological and entomological data types into a consistent framework for assessing transmission dynamics. Here we present an extension of EMOD - an individual based model of malaria transmission with human and vector agents - to include representations of parasite genetics and development. In a generic seasonal transmission setting, we simulate the spatiotemporal genetic landscape from which sequence data is sampled according to passive and active sampling methods. We then use counterfactual scenarios to illustrate what a suite of genetic signals would look like under different realistic intervention strategies. By varying the degree of human migration within and outside the region of interest, we use this model to address the relative role of importation in sustaining local transmission under different archetypal scenarios. In summary, we demonstrate the utility of parasite genetic data in characterizing malaria transmission settings and highlight the role of modeling to in guiding decision-making by National Malaria Control Programs in malaria control and elimination efforts.

1199

MODELING *PLASMODIUM FALCIPARUM* PARASITE GENETICS INSIDE AN AGENT-BASED, SPATIAL MODEL

Daniel Bridenbecker, Jonathan Russell, Albert Lee, Edward Wenger, Joshua L. Proctor, Caitlin Bever

Institute for Disease Modeling, Belleve, WA, United States

The genetics of *Plasmodium falciparum* is playing an increasingly important role in the understanding of malaria transmission and guiding the use of interventions. A model of the parasite's genetics has been integrated into IDM's open source Epidemiological MODeling software. EMOD is a stochastic, mechanistic, agent-based model that simulates the actions and interactions of individuals within geographic areas in order to understand the disease dynamics in a population over time. The new parasite genetics module takes the existing model from sporozoites to gametocytes in the human and extends it with a detailed model in the mosquito of the parasite progressing from gametocyte to oocyst to sporozoite. This enables us to more easily model parasite genome changes due to mutation and recombination. We will explain how this is modeled as well as demonstrate how different intervention strategies - such as ITN, IRS, and MDA - impact the complexity of infection as a metric of changing transmission intensity.

1200

"*WHEN WE GOT HERE, THEY WELCOMED US*": EXPERIENCES OF PRIMARY CAREGIVERS OF CEREBRAL MALARIA SURVIVORS WITH BEHAVIOR PROBLEMS ENROLLED IN THE COPS STUDY IN BLANTYRE, MALAWI

Savannah Karmen-Tuohy[1], Sebastian M. Mboma[2], John T. Langfitt[3], Rachel Brim[4], Melissa Gladstone[5], Terrie E. Taylor[2]

[1]*New York University School of Medicine, New York, NY, United States,* [2]*Blantyre Malaria Project, University of Malawi, College of Medicine, Blantyre, Malawi,* [3]*Department of Neurology, School of Medicine and Dentistry, University of Rochester, Rochester, NY, United States,* [4]*Department of Osteopathic Medical Specialties, College of Osteopathic Medicine, Michigan State University, East Lansing, MI, United States,* [5]*Department of Women and Children's Heath, Institute of Translational Medicine, University of Liverpool, Liverpool, United Kingdom*

Despite recent advances in prevention and treatment, the morbidity burden of cerebral malaria (CM) remains significant in sub-Saharan Africa, with more children surviving CM with neurodisability, including behavior problems. Little support is available for caregivers of CM survivors, and caregivers' perceptions of the few available programs are poorly characterized. We report caregivers' experiences participating in a five-year longitudinal research study in Blantyre, Malawi, including the perceived positive impact of participation and additional information or resources desired. Six focus group discussions with caregivers of CM survivors with behavior problems were conducted in July 2019. Discussions were conducted in Chichewa, audio-recorded, transcribed and translated into English for analysis. Data were manually coded, and codes were analyzed thematically. Caregivers reported the benefits of study participation to be the level of medical care provided at admission and continued care from study team clinicians, pediatric neurologists, and physical therapists throughout study enrollment. Caregivers shared perceptions of the information provided at discharge, which focused on study logistics and general advice for caring for CM survivors. Caregivers were then asked to share information they wished the study team had provided at discharge. Participants collaboratively ranked these items, creating a prioritized list of desired topics: new behaviors may emerge at different times, CM survivors will act differently than peers, specific strategies for teaching and disciplining CM survivors, and encouragement that conditions may improve. As CM-associated neurodisability emerges in the months to years following infection, caregivers benefit from longitudinal support and medical care. Caregivers are willing to participate in research, especially when studies provide care-directed education and long-term medical, rehabilitation, and social support. Future studies should consider these identified characteristics when designing programs to support and educate caregivers of CM survivors with neurodisability.

1201

LESSONS FROM IMPLEMENTING MULTI-LEVEL DATA QUALITY IMPROVEMENT INITIATIVES TO IMPROVE MALARIA DATA QUALITY IN FOUR GLOBAL FUND MALARIA PROGRAM SUPPORTED STATES IN NIGERIA

Chidinma Assumpta Okolo[1], Frank Oronsaye[1], Temitope Adeyoju[1], Nnaemeka Onugu[1], Ghislain Ismael Nana[1], Chinedu Chukwu[2], Jane Onyilo[3], Uchechukwu Ikhimioya[1], Ibrahim Maikore[4], Perpetua Uhomoibhi[4], Diwe Ekweremadu[1], Sonachi Ezeiru[1]

[1]*Catholic Relief Services, Abuja, Nigeria,* [2]*Management Sciences for Health, Abuja, Nigeria,* [3]*Malaria Consortium, Abuja, Nigeria,* [4]*National Malaria Elimination Programme, Federal Ministry of Health, Abuja, Nigeria*

Data quality plays a vital role in decision making and measuring project achievements. In Nigeria, data quality continues to be a challenge in many health projects including 2018-2020 Global Fund Malaria grant. In response, Catholic Relief Services in collaboration with National Malaria Elimination Programme, seek ways to improve data quality by rolling out multi-level data quality improvement strategies at different levels. At national level, cascade training model was used to deliver training messages from trainers at the national level to trainees at the state, local government and health facility level on the use of data collection tools. Likewise, data management SOP were printed and deployed to public health facilities, as well as automated data validation template was also developed to track and flag data errors and corrections. At the State and Local Government Area (LGA) levels, processes were established for trained personnel to conduct bimonthly LGA data validation meetings and quarterly state data review meetings as well as offer on the job mentoring to health facility staff on correct documentation and reporting. To assess improvement of data quality in 2019, data quality assessment was conducted in pre-selected health facilities in Osun, Yobe, Taraba and Gombe States using key measures of data quality. Health facility selection

criteria were based on observed recurrent poor data quality score and poor reporting rate on the District Health Information System. Result showed an average improvement for data availability, consistency and validity across the 4 States visited were 59%, 25% and 42% at baseline in 2018 and 80%, 73% and 72% respectively at post intervention. Result breakdown on data availability showed 66%, 52%, 54%, 65% to 80%, 88%, 68%, 82% in Osun, Yobe, Taraba and Gombe States respectively. Similarly, data consistency increased from 25%, 41%, 19%, 15% to 90%, 91%, 54%, 57% in Osun, Yobe, Taraba and Gombe States respectively. Data validity improved from 53%, 41%, 51%, 21% to 87%, 64%, 61%, 79%. The result shows the impact of implementation of multi-level data quality improvement initiatives on improved data quality.

1202

USE OF LAY HEALTH PERSONNEL IN THE IMPLEMENTATION OF MALARIA CASE MANAGEMENT WITHIN THE INTERNALLY DISPLACED PERSONS COMMUNITY IN NIGERIA

Uchechukwu Ikhimioya[1], Temitope Adeyoju[1], Chidinma Okolo[1], Emmanuel Shekarau[2], Nickson Hyeladendu[1], Bello A. Bello[1], Darlington Okoye[1], ThankGod Nyeche[1], Olatayo Abikoye[1], Frank Oronsaye[1], Nnenna Ogbulafor[2], Nnaemeka Onugu[1], Ghislain Nana[3], Anouk Chevalier Plante[4], Diwe Ekweremadu[1], Sonachi Ezeiru[1]

[1]*Catholic Relief Services, Abuja, Nigeria,* [2]*National Malaria Elimination Programme, Abuja, Nigeria,* [3]*Catholic Relief Services, Baltimore, MD, United States,* [4]*The Global Fund to Fight AIDS, Tuberculosis and Malaria, Geneva, Switzerland*

The crisis in North-East Nigeria caused by violent insurgency, communal clashes, and natural disasters has led to an increase in displaced persons. Over 2 million people have been displaced into camps often located in areas with environmental conditions conducive for breeding malaria vectors, creating increasing health needs for the displaced persons. This gave rise to many community-based health interventions one of which is the IDP Malaria Intervention on the 2018-2020 Global Fund Malaria grant. The intervention, implemented by Catholic Relief Services in collaboration with the National Malaria Elimination Programme, targeted 100% of IDPs in Adamawa, Gombe, Taraba, and Yobe states. The increased need to provide health services to IDPs and the existing shortage of the medical workforce, led to the identification and selection of lay health personnel in each IDP community as Community Oriented Resource Persons (CORPs). The recruited CORPs, who could read and write, were trained to identify malaria signs and symptoms, testing using Rapid Diagnostic Test (RDT), treatment, commodity and record management. A total of 1,243 CORPs were trained in clusters of 30-35 persons using practical methods. The CORPs are linked to public health facilities through Community Health Extension Workers (CHEWs) who provided continuous mentoring and supervision. A total of 468,501 IDPs with fever consulted the CORPs between December 2018 (baseline) and December 2019. Out of these fever cases, 467,198 parasitological tests (RDTs) were carried out with a cumulative testing rate of 100% (100% in Adamawa, 99% in Gombe, 100% in Taraba and 99% in Yobe states) where 352,007 tested positive and 348,949 were treated with ACTs with a cumulative treatment rate of 99% (100% Adamawa, 100% Gombe, 98% Taraba and 96% in Yobe states). This shows that CORPs have acquired skills in malaria case management. The continuous mentoring and supervision addressed the gaps in implementation. The use of lay health personnel as CORPs in IDP malaria intervention served to improve access to quality health care services and address health inequalities.

1203

ASSESSING THE REAL-WORLD STABILITY OF ARTESUNATE RECTAL CAPSULES

Hans Rietveld[1], **Andrew Slade**[1], Theodoor Visser[2], Eliza Walwyn-Jones[2]

[1]*Medicines for Malaria Venture, Geneva, Switzerland,* [2]*Clinton Health Access Initiative, Boston, MA, United States*

Artesunate rectal capsules (ARC) were initially developed and tested in large-scale clinical trials by WHO-TDR. They comprise 100mg of artesunate, in an inert lipid fill, encapsulated in a soft gel capsule. Supported by Medicines for Malaria Venture, both Cipla and Strides have developed bioequivalent products, which were prequalified in 2018 and which now play a vital part in the pre-referral treatment of severe malaria in over fifteen African countries. As part of routine product development, the stability of ARC under controlled storage conditions (25°C/60%RH, 30°C/75%RH, 40°C/75%RH) has been rigorously assessed. Globally, the data indicate that ARC is a reasonably robust product, but specifically, the data do not support 24-month shelf life under Zone IVb (30°C/75%RH) conditions. In the absence of any additional data, the shelf-life is set at 24-month under Zone II (25°C/60%RH) conditions; for clinical use, WHO-PQ recommends a cautious approach, whereby stocks of ARC should remain in field on a short-term basis for between 4 and 6 months. The need for frequent discard and resupply of ARC has proven to be logistically difficult, risks stock-outs at the community health worker level, affects trust in the product wastes limited resources. Encouraged by the data from the controlled storage studies, we will retrieve and test ARC that has been in totally un-controlled field storage for 12 months. Although the storage conditions are uncontrolled, they are not unknown. Site-specific temperature logger values, as well as local and regional maximum, minimum and average temperatures provide a comprehensive dataset against which to interpret the results of the testing. The testing itself will be done by an independent laboratory in Switzerland, using the same tests and acceptance specification as the original capsule suppliers. The data from the testing of these real-world samples, will complement the existing data from the controlled storage studies and allow end-users and regulatory agencies to make better informed decisions regarding the intervals of product replacement in the field.

1204

CLINICAL CHANGES FOLLOWING REPETITIVE CONTROLLED HUMAN MALARIA INFECTION (CHMI) IN MALARIA-NAÏVE ADULTS USING NF54 STRAIN *PLASMODIUM FALCIPARUM*

Kirsten E. Lyke[1], Andrea A. Berry[1], Patricia Ferrer-Castillo[2], Allison N. Bucsan[2], Tao Li[3], Abraham Eappen[3], Sumana Chakravarty[3], Anusha Gunesekera[3], Anita Manoj[3], Faith Pa'ahana-Brown[1], Kathy Strauss[1], Biraj Shrestha[1], Effie Nomicos[4], B. K. Sim[3], Stephen L. Hoffman[3], Kim C. Williamson[2]

[1]*University of Maryland Center for Vaccine Development and Global Health, Baltimore, MD, United States,* [2]*Uniformed Services University of the Health Sciences, Department of Microbiology and Immunology, Bethesda, MD, United States,* [3]*Sanaria, Inc., Rockville, MD, United States,* [4]*Parasitology and International Programs Branch (PIPB), HHS/NIH/NIAID/DMID, Bethesda, MD, United States*

Direct observation of acquisition of immunity is difficult in malaria field studies. A clinical study of repetitive controlled human malaria infection (CHMI) with NF54 strain *Plasmodium falciparum* via five infectious *Anopheles stephensii* mosquito bites was conducted to study clinical and immunologic effects of repeated malaria episodes. Ten core malaria-naïve adults were exposed to bites of uninfected mosquitoes with subsequent repetitive CHMI with infected mosquitoes 2, 9, 14, and 23 months later. Six additional naïve control volunteers were enrolled for CHMI 2-4, and three of these joined the repeat cohort to replace dropouts. 47 unique CHMI events occurred in 25 volunteers. Five volunteers completed four CHMIs. Parasitemia was detected by thick blood smear (TBS) and ultrasensitive PCR (usPCR) with anti-malarials administered after TBS

positivity. There was no difference in parasitemia between CHMIs. Positive usPCR preceded positive TBS by 3.55 days (range 1-8 days, P <0.0001). Pre-patent period (days) for each CHMI lengthened as assessed by TBS (CHMI1 11.46±1, CHMI2 12.22±1.9, CHMI3 13.25±1.5, CHMI4 13.8±2.4, (ANOVA test for trend P=0.007) and usPCR (CHMI1 8.09±0.3; CHMI2 8.89±1.3; CHMI3 9.38±1.8; CHMI4 9.2±1.6). No serious adverse events occurred. Notably, malaria-associated symptoms declined with successive CHMIs: For initial CHMI, 100% experienced headache, 50% fever (4 severe, i.e. >102°F), 83% malaise, and 67% myalgia (1 severe). By contrast, no volunteers developed fever in CHMI4 and only 2 experienced fever in CHMI 2 and 3. For CHMI4, the only reported malaria symptoms were moderate myalgia, headache, and malaise in one volunteer and mild malaise in another. Following CHMI1, laboratory abnormalities were 33% leukopenia (3 mild/1 moderate). Following CHMI4, 1 had mild leukopenia, 1 mild increased AST and ALT. In summary, volunteers who underwent repetitive, homologous NF54 CHMI experienced significant reduction in symptoms and prolonged pre-patency when compared with their initial malaria exposure. Future studies will focus upon immunological responses acquired over the course of repetitive CHMI.

1205

SEGMENTAL ANALYSIS ON SOCIO-BEHAVIOR NEEDS OF COMMUNITY ON MALARIA CONTROL AND ELIMINATION IN TANINTHARYI, KAYIN AND RAKHINE OF MYANMAR

Kyaw M. Tun[1], Khin T. Win[1], Zwe T. Tun[1], Mon Mon[2], Ohmar Aung[2], Ye Hein Naing[1], Sway M. Htet[1], May A. Lin[1], Alexandra T. Fischer[3], Neeraj Kak[3]

[1]*University Research Co., LLC, Yangon, Myanmar,* [2]*Independent Consultant, Yangon, Myanmar,* [3]*University Research Co., LLC, Chevy Chase, MD, United States*

Myanmar has made significant progress in reducing malaria morbidity and mortality. The number of malaria deaths has dropped steadily year by year from 1,707 in 2005 to just 19 in 2018 (about 99% reduction over 10 years) reflecting major improvements in access to early diagnosis and appropriate treatment. Recent behavior survey conducted by interviewing with 446 people from 19 villages of Tanintharyi, Kayin and Rakhine Region/States revealed behavioral gaps for malaria service provision were found varying across different segments of the community. Gender analysis revealed access to skilled health care providers (public, private, community) among male was significantly higher than those of female (P value: 0.027). Slept under LLINs in the previous night (P value: 0.002), access to skilled health providers (P value: 0.019), and knowledge on prevention of malaria by LLINs (P value: 0.002) were significantly higher among Burmese than those of other ethnicities including Rakhine, Kayin, Mon, Shan. Knowledge on prevention of malaria by LLINs, knowledge on the importance of early diagnosis and treatment of malaria, knowledge on the importance of treatment adherence, and sleeping under LLINs behavior were not significantly different between schooling at least primary level and illiterate/short-term non-formal education. Malaria control needs of the migrants could not be identified with few inclusiveness of them in the quantitative random samples. Segmental analysis on socio-behavior needs on malaria control might suggest SBCC approach should be improved to meet the needs of various social segments of the communities. For complementing quantitative findings and continuous quality improvement of Defeat Malaria socio-behavioral change communication (SBCC) activities, qualitative assessments applying key informant interviews and focus group discussions will be conducted from March-June 2020. The better intervention strategy for promoting socio-behavior aspects of key segments of communities will be explored and presented.

1206

USE OF ROUTINE SUPPLY CHAIN DATA TO IMPROVE ROLLOUT OF COMMUNITY CASE MANAGEMENT FOR MALARIA IN SIAYA COUNTY, KENYA

Miriam Okara, Felix Tindi, Lillian Gitau, Victor Sumbi, Mildred Shieshia

GHSC-PSM, Nairobi, Kenya

Malaria is a burden in most households in Kenya's Lake endemic region. A community case management (CCM) strategy for malaria in 10 counties in the region, including Siaya County, has contributed improved malaria control, with community health volunteers trained on case management and reporting into the Kenya Health Information System (KHIS). However, Siaya County's CCM commodity supply was challenged by inadequate consumption data from the 186 community units (CUs) and their "link" health facilities, resulting in frequent stockouts that affected community and facility-level programming. In 2018, the USAID Global Health Supply Chain Program-Procurement Supply Management (GHSC-PSM) project's Afya Ugavi Activity, with funding from the U.S. President's Malaria Initiative (PMI), launched a health commodities dashboard in the KHIS that includes a CU module to aggregate and analyze data from link facilities. The dashboard allows the ministry of health to measure CU supply chain performance based on reporting rate, on-time reporting, data quality and stock status; it also generates resupply quantities for CUs. During quarterly stock status and data review meetings at county and sub-county levels, health management teams use the dashboard to monitor consumption and available stock, and to identify CUs flagged for not reporting or with data quality issues. The sub-county pharmacists then visit the CU's community health extension worker to review data and provide additional training. From 2018-2020, this CU module in Siaya County has contributed to 1-Increased reporting rate for malaria commodities from 61 to 94.5% 2-Decreased stockout rate for Artemether/Lumefantrine tablets in CUs from 75 to 32% 3-Better quality CU consumption data facilitated the roll-out of CCM from 75 to all 186 CUs.By integrating a module into an existing platform rather than developing a new one, and providing funding for training, implementers can reduce duplicity and encourage use of existing supply chain tools. In return, improved data reporting and quality allow for evidence-based decisions, improving commodity availability and ensuring continuity of CCM.

1207

TEST AND TREAT; COMMUNITY OUTREACH STRATEGY IMPROVES OUTCOME IN A MALARIA UPSURGE SITUATION IN GULU DISTRICT, NORTHERN UGANDA IN 2019

Maureen Amutuhaire[1], Damian Rutazaana[1], Jimmy Opigo[1], Shirah Karungi[2], Francis Abwaimo[2], Susan Abbo[2], Susan Esther Asaasira[3]

[1]*Ministry of Health, Kampala, Uganda,* [2]*Malaria Consortium, Kampala, Uganda,* [3]*Infectious Disease Research Collaboration, Kampala, Uganda*

By 2019, Uganda made significant progress towards the reduction in malaria prevalence from 19% in 2014 to 9%. However an increased number of asymptomatic malaria cases have been detected through small surveys done within the communities of Northern Uganda. These seem to aid increased transmission of the cases. With the prolonged wet season in 2019 and wear out of the key preventive measures like Long Lasting Insecticide treated Nets, Uganda experienced an upsurge of malaria cases with some of the districts facing highest effect. Through weekly Surveillance data, Districts with the highest test positivity rates including Gulu District were identified. This study presents results from participants tested from villages that contributed highest to the burden. These were targeted through community outreaches by the Community Health workers (CHWs) through rapid diagnostic tests for malaria and treatment of the positive cases according to the Guidelines under the supervision of a health facility staff. Of the 522 people from the selected high burden villages tested, 240 (46%) participants were found positive. All were reached with health education messages on malaria prevention and

treatment significantly bringing down the district positivity rate by 11% by the end of the last quarter. CHWs once equipped, trained and supported not only identify asymptomatic malaria cases that are salient posing risk for enhanced transmission in communities but also provide response to timely contain the epidemics.

1208

CHOICE OF HEALTH SERVICE PROVIDERS AMONG THE FOREST GOER POPULATION IN MYANMAR

Si Thu Thein, Naw Eh Thi Paw, Su Su Zin, May Me Thet

Population Services International Myanmar, Yangon, Myanmar

Forest goers in the rural areas are at high risk of malaria infection, as they usually go and stay in the forest for work related activities. Our study explored health seeking behavior of forest goer population in relation to available heath service providers in their vicinity in the rural areas of Myanmar. A cross-sectional survey was conducted in 40 selected villages within 2 kilometers from the forest edge. All health care providers in each surveyed village were mapped and interviewed. At the same time, a representative sample of 479 households with forest goers were also interviewed. This approach enabled the interviewers to determine exactly which provider was chosen for health services by each household, and how far they were from each other. The choice of health care providers was analyzed by generalized linear mixed effect regression model with logit link. More than 90% of the households had access to trained volunteers and basic health staff. About half (47%) had access to private semi-formal providers (small drug shops, traditional healers, retail shops) and less than 10% to public health facilities in their village. However, only 48% of the forest goer households chose to visit any of these providers when they had fever. The rest either stayed at home and self-medicated (37%) or went to provider outside their village (15%). On regression analysis of the choice of providers in their village, we found that the forest goers were significantly more likely to choose either public health facilities (adjusted odds ratio - OR 4.1, 95% confidence interval (CI) 2.3-7.3) or trained volunteers (adjusted OR 5.7, 95% CI 3.7-8.7), compared to private semi-formal providers. On the other hand, the households were significantly more likely to choose nearby providers, as the odds increased by 6% with each one-minute reduction in walking time (adjusted OR 1.06, 95% CI 1.03-1.09). Our study approach enabled a deeper analysis on the choice of providers among forest goer population in rural areas of Myanmar. Such approach could be useful when one needs to understand the complex reasons underlying health seeking behavior.

1209

TRACKING ANTIMALARIAL RESISTANCE IN HUMANS AND MOSQUITO BLOOD MEALS: A CROSS-SECTIONAL EQUIVALENCE STUDY IN BAMA, BURKINA FASO

Hanna Y. Ehrlich[1], Anyirékun Fabrice Somé[2], Thomas Bazié[2], Ebou Cathérine Neya[2], Estelle Lotio Dembelé[2], Richard Balma[2], Martina Wade[1], Amy K. Bei[1], Brian D. Foy[3], Roch K. Dabiré[2], Jean-Bosco Ouédraogo[2], Sunil Parikh[1]

[1]*Yale University, New Haven, CT, United States,* [2]*Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso, Burkina Faso,* [3]*Colorado State University, Fort Collins, CO, United States*

Antimalarial resistance will continue to compromise current and future therapies, and effective surveillance is necessary to mitigate the spread of resistance once it has emerged. Surveillance typically relies on invasive blood samples collected from infected individuals, yet such studies are limited by costly infrastructure, regulatory oversight, and significant reporting delays. We hypothesized that mosquito blood meals could be analyzed to monitor antimalarial resistance in *Plasmodium* populations while also providing data on vector indicators. We conducted a series of cross-sectional surveys to compare resistance-associated molecular markers in humans and mosquitos in Bama, Burkina Faso. To simulate the applicability of blood meal sampling in other regions, we surveyed in two high-transmission seasons (10/2018, 09/2019) and one low-transmission season (03/2019). Household clusters (n=120) in 7 village sub-sectors were sampled proportionately to sector size. Following consent, we obtained 1,481 dried blood spots via capillary finger-prick. From the same households, we simultaneously collected 2,349 blood-fed mosquitos via vacuum aspiration and pressed abdominal contents onto FTA cards. Parasite DNA was extracted with KingFisher and *P. falciparum* (*Pf)* infections were detected with *var*ATS qPCR. Mutations were identified in *pfmdr1* N86Y, D1246Y, and *pfcrt* K76T with High Resolution Melting. Early results suggest that 81% (n=42/52) of humans and 65% (n=56/86) of mosquito blood meals were *Pf*+. Of those, 4% of both human DBS and mosquito blood meals had pure *pfmdr1* 86Y mutant infections, and 18% of humans and 14% of mosquitos had mixed N86Y infections. Using equivalence tests, we will present full statistical comparisons of the prevalence of all alleles in humans and mosquitos blood meals at each time point, at household and community cluster spatial scales, as well as comparative frequency data with multiplicity of infection. Our study is sufficiently powered to validate or invalidate the use of mosquito blood meals as a low-cost and rapidly deployable tool to monitor changes in the distribution of antimalarial resistance.

1210

VIRAL HEPATITIS AND MALARIA CO-INFECTION IN THE DEMOCRATIC REPUBLIC OF THE CONGO (DRC)

Emily N. Reichert[1], Peyton Thompson[1], Cedar L. Mitchell[1], Nicholas F. Brazeau[1], Molly Deutsch-Feldman[1], Kyaw L. Thwai[1], Gavin Cloherty[2], Jonathan J. Juliano[1], Steven R. Meshnick[1], Jonathan B. Parr[1]

[1]*University of North Carolina, Chapel Hill, NC, United States,* [2]*Abbott Laboratories, Abbott Park, IL, United States*

Hepatitis B virus (HBV), hepatitis C virus (HCV) and malaria affect the liver and are co-endemic throughout regions of Africa. While there are no well-established associations between these infections, genetic variants within and near the human interferon lambda four (IFNL4) gene have been associated with clearance of HCV and African ancestry. These findings motivated us to evaluate associations between viral hepatitis and malaria in a well-characterized sample set drawn from the nationally representative 2013-2014 Democratic Republic of the Congo (DRC) Demographic and Health Survey (DHS). We evaluated 2,232 subjects who had undergone testing for both viral hepatitis and malaria, including species-specific malaria real-time PCR assays for *Plasmodium falciparum* (*Pf*), *P. vivax* (*Pv*), and *P. ovale* (*Po*); Abbott RealTime HCV RNA and/or ARCHITECT HBV surface antigen (HBsAg) testing. Among 679 adults tested for HBsAg, the frequency of HBV infection among those positive for *Pf, Pv,* or *Po* malaria and non-malarious subjects was similar (5.6% [95% CI 3.2-9.4] *vs* 3.7% [2.2-6.1]; p = .25). Similarly, of 1,276 adults tested, while HCV infection was seen at a higher proportion in adults with *Pf, Pv,* or *Po* malaria, no clear association emerged (1.9% [0.9-3.9] *vs* 0.8% [0.3-1.7]; p = .10). However, among 277 children <5 years of age tested for HBsAg, there was evidence for a higher HBV prevalence among those infected with *Pf* (6.8% [3.0-14.0] *vs* 1.1% [0.2-4.5]; p=.01). In summary, viral hepatitis-malaria co-infection was common in this cohort. While HBV was more common among children with falciparum malaria, we did not observe clear evidence of associations between hepatitis and malaria across age strata. Studies that consider co-infection by these endemic, high-burden infections in countries like the DRC are important both to understand any pathogen interactions and to inform the design of efficient, cross-cutting intervention programs. Further studies are needed to improve our understanding of the observed association between HBV and malaria in children.

1211

DATA QUALITY ASSURANCE IN HEALTH FACILITIES IN THREE HEALTH DISTRICTS OF BURKINA FASO

Siaka Debe[1], David Kangoye[1], René Kinda[1], Adama Ganou[1], Cheick S. Compaoré[2], N'Fale Sagnon[1], Moussa Guelbeogo[1], Christelle Gogue[3], Adama Gansané[1], Molly Robertson[3]

[1]*Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso,* [2]*Programme National de Lutte contre le Paludisme, Ouagadougou, Burkina Faso,* [3]*PATH, Washington, DC, United States*

The effectiveness of malaria vector control tools is threatened by the emergence and intensification of insecticide resistance in mosquito populations. Next-generation insecticide-treated bednets effective against pyrethroid-resistant mosquitoes have been developed, including the Interceptor® G2 (BASF) (IG2). Nets with a pyrethroid and the insecticide synergist piperonyl butoxide have also been made available. As part of the New Nets Project (funded by a partnership between Unitaid and The Global Fund), observational studies are accompanying a piloted IG2 rollout in order to collect data on their entomological and epidemiological impact and on anthropological factors that influence their uptake and usage. The epidemiological component of the pilot includes malaria case data recorded by health centers and reported through the national health information system. To enhance the quality of these data, data quality assessments (DQA) are conducted routinely in the study districts. In Burkina Faso, the first DQA covered malaria data recorded in May 2019 in the three study districts, Banfora, Gaoua, and Orodara. In each district 35% of health facilities (e.g., health posts and hospitals) were randomly selected for the evaluation. The DQA focused on validity, completeness, and concordance for 12 malaria indicators. These indicators, which are reported monthly, were extracted from *District Health Information Software* (DHIS2) and compared to the source data comprised of health facility registers and reports. A total of 55 health facilities were included in this first assessment. Completeness for the 12 indicators was 100%. No indicator was reported with 100% precision in DHIS2, ranging from 90% for 8 indicators, to 70-90% for 2 indicators, and less than 70% for 2 indicators. Presumptive malaria and mortality rates were underestimated while the 10 remaining indicators were overestimated in DHIS2. Overall, data quality on malaria indicators in Burkina Faso is moderate. There is a need to enhance competencies and capacities of health workers to improve the quality of the health data for effective decision-making.

1212

HEALTHCARE PROVIDERS' PRACTICES ON MALARIA RDT MANAGEMENT IN HEALTH FACILITIES IN NIGERIA: AN EXPLORATORY STUDY

Sonachi Ezeiru[1], Diwe Ekweremadu[2], Temitope Ipinmoye[2], Victor Adebayo[2], Olatayo Abikoye[2], Genevieve Eke[3], Ezinne Onuba[2], Chukwudi Uche[3], Bala M. Audu[4], Wellington A. Oyibo[5]

[1]*Catholic Relief Services (CRS)(Principal Recipient of the Global Fund, Malaria in Nigeria), Federal Capital Territory, Abuja, Nigeria,* [2]*Catholic Relief Services, Abuja, Nigeria,* [3]*Independent Consultant, Abuja, Nigeria,* [4]*National Malaria Control Programme, Federal Ministry of Health, Abuja, Nigeria,* [5]*College of Medicine of the University of Lagos, Lagos, Nigeria*

Current malaria case management practices require mandatory parasitological confirmation of all suspected malaria cases with microscopy or malaria Rapid Diagnostic Tests (RDTs). Malaria RDTs are to be stored appropriately in health facilities to avoid likely degradation of the monoclonal antibodies on the nitrocellulose strips in the RDT cassettes when exposed consistently to high environmental temperature. This exposure could affect the performance of the RDTs with attendant misdiagnosis of patient, loss of confidence on RDTs and ultimately rejection of RDT use. Malaria RDT is critical in expanding access to parasitological confirmation, especially in majority of the Primary healthcare (PHC) facilities where laboratory testing is not accessible. We assessed healthcare providers' awareness, perception and practices on malaria RDT storage in health facilities (HFs) in Delta, Kaduna and Ogun state, Nigeria. A total of 198 HFs in the three states: 25 HFs in Delta (12.6%), Kaduna, 138 (69.7%) and Ogun 35(17.7%) were visited for this study. Malaria RDTs were introduced in these facilities in the last 2-10 years, and average of 6 years per HF. Storage practices for malaria RDTs varied across HFs. 85.2% of the HCPs in the HFs reported RDTs were kept together with medicines; 25.8% of the HCPs reported that RDTs were kept in separate rooms from medicines, while 24% reported RDTs were kept in laboratories and managed by the lab (25.8%); 14.6% in Head of Labs' Office; 17.7% in Nurses/CHEWs' Office, and 6.1% on the floor in cartons in Junk/unused rooms. Dedicated storage shelves for medicines and RDT was seen in 62.1% of the HFs while dedicated shelf for RDTs alone was seen in 47% of the HFs. Air conditioning in storage room was available in 33.4% of the HFs. However, room thermometer for temperature monitoring was only available in 7.1% HFs assessed; and of these temperature monitoring records was availablein 4.5% HFs visited. Stock records for medicines and RDTs was seen in 88.8% and 72% of HFs respectively. Data on stock-out of RDTs was available in 35% of these facilities. We recommend that RDTs be stored along with medicines and temperature monitoring instituted.

1213

THE QUALITY OF CASE MANAGEMENT FOR SEVERE MALARIA EPISODES AND ITS IMPACT ON THE HEALTH OUTCOMES IN REMOTE ENDEMIC SETTINGS OF THE DEMOCRATIC REPUBLIC OF CONGO

Jean Okitawutshu[1], Antoinette Tshefu[1], Jean-Claude Kalenga[1], Aita Signorell[2], Manuel Hetzel[2], Christian Burri[2], Nina Brunner[2], Giulia Delvento[2], Theodoor Visser[3], Valentina Buj[4], Christian Lengeler[2]

[1]*Ecole de Santé Publique, Université de Kinshasa, Kinshasa, Democratic Republic of the Congo,* [2]*Swiss Tropical and Public Health Institute, University of Basel, Basel, Switzerland,* [3]*Clinton Health Access Initiative, Boston, MA, United States,* [4]*UNICEF, New York, NY, United States*

Malaria is still a major cause of morbidity and mortality globally, being the third highest infectious disease killer of children under 5 years of age. Rectal Artesunate (RAS) is recommended as part of the integrated community case management (iCCM) algorithm as a pre-referral treatment followed by referral to a higher-level health facility for a full treatment course for severe malaria. However, field-level evidence of a child's evolution and health outcome post-discharge after an episode of severe malaria is sparse. The results presented here are part of an ongoing study on Community Access of Rectal Artesunate for Malaria (CARAMAL). Aim: The purpose of this investigation is (1) to describe health outcomes 28 days after the patients first sought care at community level where they would receive one dose of RAS (community health workers and primary health facilities) compared to children directly attending referral facilities, and (2) to assess the quality of severe malaria case management and explore its impact on health outcomes. A patient surveillance system was set up in three rural Health Zones in DRC (Ipamu, Kenge and Kingandu) to enroll children <5 years with suspected severe malaria seeking care at community level, primary and secondary health facilities. The children were followed for 28 days from first contact with the healthcare system to track health status and diagnosis, pre- and post-referral treatment and health outcomes. From July 2018 to March 2019 (before RAS roll-out), 612 children < 5 years were enrolled in the study. Malaria was confirmed via diagnosis (either mRDT or thick blood smear) in 98.4% of the enrolled children. Half of the children (46.1%) had already taken an antimalarial prior to referral. Post-referral treatment consisted of quinine in half of the children, 1/3 were treated with injectable artesunate and 18.8% received antibiotics. On day 28, 16.3 % were still reported to be sick, 51.5% had a positive RDT, 6.4% had severe anemia. The findings suggest that the quality of treatment overall is poor, contributing to poor health outcomes.

1214

READINESS OF FORMAL PRIVATE SECTOR PROVIDERS FOR MALARIA CASE MANAGEMENT IN MALARIA HIGH BURDEN AREAS OF MYANMAR

Ye Kyaw Aung, Si Thu Thein

Population Services International, Yangon, Myanmar

Population Services International (PSI) Myanmar has been engaging with formal private sector providers (clinics and pharmacies) in malaria high-burden areas in Myanmar to test, treat, and report malaria cases. This study aimed to evaluate such providers' readiness for malaria case management in those high-burden areas. A quantitative cross-sectional survey was conducted in 17 malaria high-burden townships during September 2019. A full census approach was applied to identify all providers with potential to distribute antimalarials and/or malaria testing in the selected areas. If they stocked any antimalarials or any malaria test on the day of the survey or within the previous 3 months, an audit of all antimalarials and malaria rapid diagnostic tests (RDT) and a provider interview was conducted. Data analysis was performed in STATA 14.2. As the study consisted of all identified providers in the survey area (241 clinics and 373 pharmacies), the majority were not affiliated to PSI (85.9% of clinics and 91.7% pharmacies). Overall, about 50% of clinics and 30.7% of pharmacies stocked at least one antimalarial medicine at the time of the survey. However, only 22.3% of clinics and 2.4% of pharmacies stocked both RDT and first-line artemisinin combination therapy (ACT). Only 11.5% of clinics and 4.8% of pharmacies had at least one recently trained provider while 45.7% and 4.4% had practices of record keeping and reporting of malaria case management, respectively. On readiness for malaria case management, PSI affiliated private sector providers fared better than non-affiliated ones (Stock of both RDT and ACT: 47.7% vs 17.2% for clinics and 59.6% vs 1.0% for pharmacies, Malaria case reporting: 89.3% vs. 31.7% for clinics and 65.6% vs. 0.5% for pharmacies). In malaria high burden areas of Myanmar, a large number of formal private sector providers kept antimalarial medicines, but they were not ready for proper malaria case management and reporting. Proper support of such providers could change their practice and would be helpful for malaria control and elimination.

1215

ADHERENCE TO PRENATAL MALARIA PREVENTION PRESCRIPTION AND USE OF INSECTICIDE TREATED BED NETS BY PREGNANT WOMEN IN ENUGU NIGERIA

Chikere A. Anusiem[1], Michael I. Nwafor[1], Chigorom A. Anusiem[2], Chigozie P. Okorie[1]

[1]*College of Medicine University of Nigeria Teaching Hospital Enugu, Enugu, Nigeria,* [2]*University of Nigeria Teaching Hospital Enugu, Enugu, Nigeria*

Adherence to medical prescription and other advice given by healthcare givers to pregnant women during prenatal clinic visits is vital in achieving the goals of maintaining good health during pregnancy and having healthy newborn baby. Malaria in pregnancy is major contributor to morbidity and mortality in pregnant mothers and their unborn babies. Intermittent preventive treatment of malaria for pregnant women (IPTp) using tablets of sulfadoxine-pyrimethamine (S-P) is a key strategy recommended for prevention of malaria during pregnancy in Nigeria. The use of insecticide treated bed nets (ITN) to prevent malaria parasite-transmitting mosquitoes from biting the users is another key strategy for reducing the incidence of malaria. Pregnant women are advised to use insecticide treated bed nets (ITN) regularly. We carried out a cross-sectional study to investigate the extent of adherence of pregnant women to their prescribed antimalarial tablets (S_P) and to the recommended use of ITN as well as the impact of their socio-demographic (personal) characteristics on their adherence profiles. Pre-validated questionnaires were completed in three busy antenatal clinics located in Enugu town in eastern Nigeria by all consenting pregnant women who visited any of those clinics for prenatal care during the period of the study. The clinics were chosen by stratified random sampling. Data analysis was done using SPSS version 20, New York). Statistical differences were determined at $p < 0.05$. The proportion of respondents who adhered strictly to their antenatal clinic prescription for malaria prevention using S-P was 76.9% (166) and non-adherence observed was 23.1% (50). Among the respondents who used ITN 78.4% (98 respondents) used ITN every night, while 57.9% used ITN but not every night, and 42.1% did not use it. There positive association of use of ITN with increasing maternal age, level of education, and number of previous childbirths.

1216

IMPROVING QUALITY OF CARE (QOC) IN MALAWI THROUGH OUTREACH TRAINING AND SUPPORTIVE SUPERVISION (OTSS)

John Munthali[1], Dubualo Moyo[2], Kate Wright[3], Tom Hall[4]

[1]*Management Sciences for Health, Lilongwe, Malawi,* [2]*Ministry of Health, Malawi, Lilongwe, Malawi,* [3]*Management Sciences for Health, Medford, Malawi,* [4]*Management Sciences for Health, Arlington, VA, United States*

The World Health Organization identifies Quality of Care (QoC) as an essential pillar to achieving Universal Health Coverage for women, children, adolescents and the wider population. In collaboration with Malawi's National Malaria Control Program (NMCP), ONSE Healthy Activity, with funding from the President's Malaria Initiative, is using a tablet-based supervision tool - outreach training and supportive supervision (OTSS) - to strengthen malaria QoC for a more effective and efficient health system. OTSS is a strategic approach that is part of a broader quality assurance system and encourages two-way communication between facility staff and supervisors to improve performance. Globally, OTSS tracks six indicators while Malawi added three severe malaria indicators, for a total of nine. Between 2017 and 2020, the ONSE Health Activity supported 1,130 OTSS visits to 226 health facilities using an NMCP-approved, standardized checklist. The checklist specifically addresses malaria clinical and diagnostic services, with the aim of improving malaria case management, including laboratory, pharmacy, and outpatient components. The use of tablets allows for automatic upload of OTSS data into DHIS2 for immediate access and analysis. Performance improvement is measured through tracking of nine performance indicators including for diagnosis, treatment (including severe cases), and data for decision-making. Improvements related to competency and adherence were achieved in all indicators from 70% in 2017, 77% in 2018, 82% in 2019 to 89% in 2020, consistent with an upward trend in performance. These consistent, annual, averaged improvements for these nine indicators show that a well-designed and implemented OTSS program provides a platform for ongoing support to strengthen clinical and diagnostic services in health facilities by identifying improvement needs in knowledge and skills and providing support to clinicians, data, and laboratory staff to address these needs. An effectively implemented OTSS strengthens the links between diagnostic and clinical services, assuring the appropriate care is available, thus improving QoC.

1217

EFFECT OF THE IMPLEMENTATION OF SEASONAL MALARIA CHEMOPREVENTION (SMC) ON THE AVERAGE MONTHLY CONSUMPTION OF RDTS IN TWO HEALTH DISTRICTS IN NORTHERN BENIN IN 2019: MK (MALANVILLE-KARIMAMA) AND TMC (TANGUIETA-MATERIAL-COBLY)

Mario Badet, Vivien Akan, Cyriaque Dossou Affoukou, Aurore Ogouyemi-Hounto

Programme Notional de Lutte contre le Paludisme, Cotonou, Benin

Seasonal Malaria Chemoprevention (SMC) is a World Health Organization-recommended strategy that aims at reducing malaria-related incidence among children aged 3 to 59 months. A study was conducted to measure the impact of this strategy on the average monthly consumption (AMC) of rapid diagnostic tests (RDTs). We assumed that a decrease in the AMC would be in direct link with a decrease in the number of malaria cases. The study compared on the one hand the AMC of RDTs in SMC districts Malanville-Karimama (MK) and Tanguieta-Material-Cobly (TMC) for 2018

and 2019 over the months corresponding to SMC implementation period (July to October). On the other hand, RDTs' AMC at the districts that benefited from SMC was compared with control that are geographically contiguous in Kandi Gogounou Segbana (KGS) and Natitingou Boukombé Toucountouna (NBT). The average completeness of logistics reports in the 4 study districts is 91.75%. Student's T-test was used for the comparisons. Comparative analysis of data showed a reduction of 21.66% and 3% in AMC in TMC and MK respectively (SMC area) from July to October (19102 in 2018 and 14963 in 2019 in TMC; 6501 in 2018 and 6311 in 2019 in MK). Results showed a 15% and 98% increase in the RDTs AMC in NBT and KGS respectively (non-SMC area) from July to October (14951 in 2018 and 17160 in 2019 in NBT; 6858 in 2018 and 13582 in 2019 in KGS). SMC resulted in a statistically significant decrease RDTs' AMC in MK (SMC area) compared to that of KGS (non-SMC area that is geographically close), *p value = 0.001 (13582 in KGS and 6311 in MK during the same period*). SMC reduced RDT consumption which could likely be a result of a decrease in the reported malaria cases in the intervention areas. The results of this study point to the need for this approach be continued and expanded to other eligible health districts to contribute to other malaria control interventions impact on morbidity and mortality in children aged 3 to 59 months.

1218

COMMUNITY PERCEPTIONS OF *PLASMODIUM FALCIPARUM* MALARIA RISK AND USE OF PREVENTION IN SUSSUNDENGA, MOZAMBIQUE

Keeley J. Morris[1], João L. Ferrão[2], Dominique Earland[1], Anísio Novela[3], Albino Bibe[4], Kelly M. Searle[1]

[1]*University of Minnesota, Minneapolis, MN, United States,* [2]*Instituto Superior de Ciências de Educação, Beira, Mozambique,* [3]*Direcção Distrital de Saúde de Sussundenga, Manica, Sussundenga, Mozambique,* [4]*Escola Secondária de Sussundenga, Sussundenga, Mozambique*

Across south-east Africa, many countries have documented substantial declines in incidence, morbidity, and mortality due to *Plasmodium falciparum* malaria over the past 20 years. These successes have not been universal, with heterogeneity within and across countries. Mozambique continues to have the 5th highest *P. falciparum* prevalence globally and 3rd highest in Africa. The highest incidence of *P. falciparum* malaria in Mozambique occurs in the central and northern regions. We used satellite imagery to identify and enroll a random sample of 100 households in rural Sussundenga, Manica Province in central Mozambique along the Zimbabwe border. We administered a survey to residents of enrolled households to collect information on knowledge about malaria, perceptions of malaria risk, and use of malaria prevention. Ninety-three households had at least 1 member complete the survey. Surveys were collected from any consenting member of an enrolled household (adult or child) and 358 residents completed the study. Initial analyses indicate that 35% (n=357, 95% CI [29.9%-39.8%]) of participants report not using insecticide-treated bed nets (ITN) the preceding night. Eighty-two percent (n=124, 95% CI [74.4% - 88.1%]) of those not using ITNs indicated lack of access or inability to hang the ITN as the primary reason for lack of use. We also asked participants questions related to their knowledge about malaria transmission and their perception of malaria risk at the individual, family, and community level using a 4-point Likert scale. Preliminary analysis of participant reported risk found that 81% (n=274, 95% [76.3%, 85.6%]) of respondents said they "disagree" or "strongly disagree" with the statement that, "young people are at higher risk for malaria". We will compare knowledge about malaria transmission and perceptions of risk to ITN use to evaluate potential motivating factors for use of malaria preventatives in this area. Our results will provide important epidemiologic and health behavior information about a region experiencing persistent malaria endemicity and will inform future implementation of control interventions.

1219

COVERAGE, USE, AND IMPACTS OF *PLASMODIUM FALCIPARUM* MALARIA PREVENTION AND CONTROL MEASURES IN RURAL SUSSUNDENGA, MOZAMBIQUE

Kelly M. Searle[1], Dominique Earland[1], Albino Bibe[2], Anísio Novela[3], João L. Ferrão[4]

[1]*University of Minnesota, Minneapolis, MN, United States,* [2]*Escola Secondária de Sussundenga, Sussundenga, Mozambique,* [3]*Direcção Distrital de Saúde de Sussundenga, Manica, Sussundenga, Mozambique,* [4]*Instituto Superior de Ciências de Educação, Beira, Mozambique*

With many countries across sub-Saharan Africa documenting substantial declines in *P. falciparum* malaria, Mozambique has made limited progress towards control. Malaria remains among the leading causes of morbidity and mortality in Mozambique with the 5th highest prevalence in the world and 3rd highest prevalence in Africa. Few studies have focused on *P. falciparum* epidemiology and transmission dynamics in central Mozambique, which is of particular interest due to its proximity to Zimbabwe and its importance for regional malaria control and elimination. Sussundenga is a rural village in Manica Province that lies along the Zimbabwe border, making evaluation of transmission and control policies integral for regional efforts. The objective of this analysis was to determine the *P. falciparum* community prevalence and estimate the coverage, use, and impacts of current interventions. We conducted a cross-sectional community-based survey from December 2019 - February 2020. We used a random household sampling method, based on enumerated households from satellite imagery. All participants completed a survey about malaria prevention, interventions, and received a *P. falciparum* malaria rapid diagnostic test (RDT). The prevention and interventions strategies that were assessed were use of ITNs (insecticide treated nets), IRS (indoor residual spraying), MDA (mass drug administration), and RCD (reactive case detection). We enrolled 96 households with 358 individuals. The *P. falciparum* prevalence was 31.6% (95% CI [26.6-36.5]). We analyzed these data using a generalized estimating equations (GEE) logistic regression model to account for clustering of household variables. IRS, MDA, and RCD were largely absent in this area. Our findings indicate moderate use of ITNs that is limited by access and coverage. ITN use was associated with a decreased risk for *P. falciparum* infection. This area has limited malaria control interventions and locally these findings can inform targeting of interventions within this area. Overall, these findings can be used to enhance efforts toward regional malaria control and elimination efforts.

1220

OWNERSHIP, USE AND EFFICACY OF LONG-LASTING INSECTICIDAL NET IN PREGNANT WOMEN, A NATIONAL SCALE STUDY IN BENIN

Armel Djenontin[1], Aziz Bouraima[2], Christophe Soares[2], Marc Fiogbe[3], Gilles Cottrell[4]

[1]*Université d'Abomey-Calavi, Faculté des Sciences et Techniques/Centre de Recherche Entomologique de Cotonou, Cotonou, Benin,* [2]*Centre de Recherche Entomologique de Cotonou, Cotonou, Benin,* [3]*Institut de Recherche pour le Développent, Cotonou, Benin,* [4]*Université de Paris / Institut de Recherche pour le Développement, Cotonou, Benin*

Malaria infection during pregnancy has serious consequences for the health of the mother and the newborn's birth weight. During pregnancy, the long-lasting insecticidal net (LLIN) is one of the main malaria prevention tool recommended by the World Health Organization (WHO). In Benin, according to national policy, the pregnant women should be given a LLIN at their first antenatal visit. However, the proportion of pregnant women who will be using these LLINs is unknown. Therefore, there is a lack of knowledge about the actual ownership, use and efficacy of LLINs in pregnant women on the field. To assess this issue, we carried out a national scale study in Benin. From November 2019 to September 2020, we enrolled 720 pregnant women at their first antenatal visit in 3 sites (South, Center, North) of the country, in both urban and rural areas.

They were visited twice at home one month apart during their pregnancy to evaluate the ownership and use, and in the laboratory, the bio efficacy and physical integrity of all pregnant women's LLIN were evaluated. Up to date, 549 pregnant women were visited twice. Among them, 97% declared to be owning a LLIN which were seen on the sleeping units of 94% of them. The proportion of pregnant women who received a LLIN at the first antenatal visit was 69%, out of which only 14% were used by the pregnant women (seen on the sleeping units). The physical integrity has been evaluated to be good for 61% of the LLIN, and 50% of the tested LLIN reached the WHO threshold of the bio-efficacy. These preliminary results show that a vast majority of pregnant women do not use the LLIN received at the first antenatal visit, but rather a LLIN previously acquired. Therefore, the physical integrity and the bio-efficacy of the bed nets used by the pregnant women are sub-optimal in a high proportion. Those results should be taken into account by the health authorities in order to optimize the malaria control policy in pregnant women in Benin.

1221

IMPACT OF A LONG-LASTING INSECTICIDE TREATED NET DISTRIBUTION CAMPAIGN IN GRANDE'ANSE AND SUD DEPARTMENTS OF HAITI

Will T. Eaton[1], Lotus L. van den Hoogen[1], Alyssa Young[2], Matt Worges[1], Phoebe Meyer[3], Ruth A. Ashton[1], Thomas Druetz[4], Wilmar Belizaire[3], Thom P. Eisele[1]

[1]*Center for Applied Malaria Research and Evaluation, Tulane School of Public Health and Tropical Medicine, New Orleans, LA, United States,* [2]*Clinton Health Access Initiative, Boston, MA, United States,* [3]*Clinton Health Access Initiative, Port-au-Prince, Haiti,* [4]*Department of Social and Preventive Medicine, University of Montreal School of Public Health, Montreal, LA, Canada*

Haiti is one of only two countries in the Caribbean with sustained transmission of *P. falciparum* malaria. The Malaria Zero consortium are supporting the National Malaria Program to eliminate malaria from Haiti by 2025. A November 2017 long-lasting insecticide treated net (LLIN) distribution campaign was among the supported interventions within a region with historically low LLIN coverage. There is limited and conflicting evidence on the effectiveness of LLINs against the primary exophilic malaria vector, *An. albimanus*. We evaluated the impact of the LLIN distribution campaign on malaria incidence in the Grand'Anse and Sud departments using an interrupted time series (ITS) design. The pre-intervention period was defined as January 2015 through October 2017 and the post-intervention period from January to December 2018. The analysis was stratified by department and included 44 health facilities within the Grand'Anse and 54 within the Sud department. From 2017 to 2018, annual malaria incidence decreased from 18.1 to 6.1 cases per 1,000 and 7.2 to 3.2 cases per 1,000 population in the Grand'Anse and Sud departments, respectively. The outcome indicator of monthly confirmed malaria case counts was based on passively detected, routine data. Results from a mixed effects negative binomial regression (with health facility as the random effect) revealed that the Grand'Anse post-intervention period experienced a proximate 64% decline (level change) in the malaria incidence rate (IRR = 0.359, 95% CI: 0.285-0.453, p <0.001) compared to the pre-intervention period after accounting for seasonality, climate, and environmental variables. Meanwhile, the Sud department experienced a 33% decline in malaria incidence rates (IRR = 0.670, 95% CI: 0.502-0.894, p < 0.001). There was evidence of a largely sustained effect in malaria incidence compared to pre-LLIN campaign trend in both settings. We now have 2019 case data available to assess this post-intervention trend over a longer time period. Results suggest that the November 2017 LLIN distribution campaign within the Grand'Anse and Sud departments had a significant effect on malaria incidence rates.

1222

UNDERSTANDING THE FEASIBILITY AND ACCEPTABILITY OF EXTENDING DELIVERY OF SEASONAL MALARIA CHEMOPREVENTION TO CHILDREN AGED FIVE TO TEN YEARS IN CHAD

Zana Coulibaly[1], Azoukalne Moukenet1, Kevin Baker2, Beakgoube Honore1, Charlotte Ward2

1Malaria Consortium, Chad, 2Malaria Consortium, UK.

Delivering treatment to children aged 3–59 months is the current approved protocol for seasonal malaria chemoprevention (SMC) administration in Chad. However, coverage data suggest that SMC is often incorrectly administered to children older than five. In parallel, there is also a global discussion about extending the eligible age range of SMC to provide protection to children up to ten years. A qualitative study was conducted between February and March 2020, in the rural and urban areas of Massaguet health district, to assess the feasibility and acceptability of extending SMC to an older age group. Eight focus group discussions (FGDs) were held in three rural villages and one urban settlement with community health workers and caregivers. Men and women participated in separate FGDs, and women were split into older (>30 years) and younger (≤30 years) age groups. Fifteen key informant interviews were held with stakeholders – including religious and community leaders in the community, health facility and district health personnel, and the malaria program and drug authority at national level. Topic guides were based on five dimensions of implementation defined by the UK Medical Research Council's framework for process evaluation of complex interventions: implementation process, fidelity, adaptations, dose and reach. Local Arabic or French was used to conduct the FGDs or interviews. Completed in March 2020, data analysis is underway and the results of this study will be ready for presentation. Findings should help us to better understand if SMC extension to elder age group is perceived to be an effective measure to protect more children against malaria, and therefore likely to be well accepted by communities and health workers.

1223

THE USEFULNESS OF SENSITIVE MOLECULAR TOOLS AND SUITABLE GUIDELINES FOR AVOIDING THE RISK OF TRANSFUSION-TRANSMITTED MALARIA

Mariana Aschar[1], Sandra C. Montebello[2], Alfredo Mendrone-Junior[2], Maria L. Farinas[1], Maria J. Costa-Nascimento[3], Jose E. Levi[1], Silvia M. Di Santi[1]

[1]*Sao Paulo University, Sao Paulo, Brazil,* [2]*Fundacao Pro Sangue Hemocentro of Sao Paulo, Sao Paulo, Brazil,* [3]*SUCEN, Sao Paulo, Brazil*

Asymptomatic malaria has been reported in blood donors worldwide. According to the Brazilian guidelines, candidates for blood donors are routinely screened for detection of *Plasmodium* or its antigens if they are residents or had displacement to an endemic area after 30 days and within 12 months of arrival. If this displacement occurred with less than 30 days, the donation is not allowed. The most used tests are the thick blood smear, the gold standard in Brazil and the RDTs, whose sensitivities are not suitable for detecting low parasitemia. Earlier this year, *Plasmodium malariae* was detected in a blood donor candidate at Fundacao Pro-Sangue Hemocentro of Sao Paulo, the reference blood center of WHO in the state. The candidate traveled to Cuiaba, a city located in an endemic region (Mato Grosso/Brazil) ten months ago. The thick blood smear reveled only one form suggesting *Plasmodium*, leaving doubt in the microscopist. A TaqMan® (ThermoFisher) qPCR in house was performed to confirm the result, which detects *Plasmodium* (2 parasites/µL). The Screen & Type® multiplex qPCR (altona Diagnostics) for the five *Plasmodium* species was used, resulting in *Plasmodium malariae*. In the epidemiological survey, the candidate referred usually travels to the city of Paraibuna, located in the Atlantic Forest in the state of Sao Paulo, where asymptomatic cases have been described. Even before qPCR results the blood unit was blocked by the blood bank. The candidate was treated and followed up according to Brazilian guidelines. This case highlights the need for more rigorous

screening and sensitive methods for detection of *Plasmodium* in blood banks, especially in non-endemic regions. If no molecular diagnosis were available, the outcome would be a case of transfusion-transmitted malaria due to an asymptomatic donor with displacement to a low transmission area. To avoid these incidents, the guidelines should consider displacement to regions where rare autochthonous cases are reported, mainly those linked to close contact with forest. In a broad context, sensitive tools are needed for the diagnosis of malaria, still a challenge for control and elimination efforts.

1224

ANTENATAL CARE VISITS AND INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY UPTAKE GAP IN LIBERIA

Jethro Zawolo[1], Birhanu Getahun[1], Gaspar Mbita[1], Julius Gilayeneh[2], Maweyata Sow[1], Gladys Tetteh[3], Lolade Oseni[3], Lauretta Nagbe[1], Anne Fiedler[1]

[1]*USAID Strategic Technical Assistance for Health (STAIP), Monrovia, Liberia,* [2]*National Malaria Control Program (NMCP) Ministry of Health (MOH), Monrovia, Liberia,* [3]*Jhpiego Johns Hopkins University, Baltimore, MD, United States*

According to the National Malaria Control Program (NMCP) guidelines, adopted from the World Health Organization (WHO) 2012 recommendations for Intermittent Preventive Treatment in pregnancy (IPTp), IPTp administration in Liberia starts at thirteen weeks gestation and is given once a month until delivery, with the expectation that at the fourth antenatal care visit (ANC4), a pregnant woman should have received at least three doses. The NMCP started the nationwide implementation of the WHO recommendations for IPTp in 2013. In 2016, 79% of pregnant women in Liberia had four or more ANC visits and 22% had at least three IPTp doses (IPTp3+). In 2019, STAIP project analyzed routine surveillance data from the District Health Information System (DHIS-2) to monitor ANC4 and IPTp3 coverage trends and determine the proportion of pregnant women who attended at least four ANC visits and received IPTp3 in 2017-2019. We used a two-population z-test to compare ANC4 and IPTp3 coverage and calculated the coverage gap as ANC4/ANC1-IPTp3/ANC1. In 2017, 2018 and 2019, the ANC4 coverage was 47%, 45% and 46%, respectively while IPTp3 was 10%, 15% and 28% respectively, yielding coverage gaps of 37%, 30% and 18% respectively. From 2017 to 2019, the ANC 4-IPTp3 gap was reduced by 19% (proportion19%; 95% CI: 17-21%; p<0.0001; z=28.01). This is likely a result of the adoption and implementation of the new IPTp3+ recommendation. The ANC4-IPTp3 gap has narrowed over 3 years from 37% to 18%. Still, over a third of women who attended at least four ANC visits in 2019 did not receive IPTp3. This assessment found a high coverage gap between ANC4 visit and IPTp3 uptake. The NMCP and its partners will investigate the causes of the ANC4-IPTp3 gap and institute appropriate interventions to address the missed opportunities.

1225

MEASURING THE IMPACT OF SEASONAL MALARIA CHEMOPREVENTION USING PROPENSITY SCORE MATCHING FOR COUNTERFACTUALS IN FOUR MALARIA-BURDENED STATES IN NIGERIA (2017-2019)

Taiwo Ibinaiye[1], Monica Anna de Cola[2], Sol Richardson[2], Olatunde Adesoro[1], Chinwe Nweze[3], Olusola Oresanya[1]

[1]*Malaria Consortium, Abuja, Nigeria,* [2]*Malaria Consortium, London, United Kingdom,* [3]*University of Nigeria Nsuka, Nsuka, Nigeria*

Clinical trials have shown that seasonal malaria chemoprevention (SMC) is a safe and efficacious intervention, preventing up to 75% of malaria episodes. Using routinely collected data is often considered the most feasible approach for assessing impact of large-scale public health programs. However, estimates of SMC impact using routine data have shown lower effect sizes than expected. This may be due to issues in data quality or lack of control (no SMC) areas after rapid scale up of SMC. We therefore, selected matched intervention (SMC) and control sites that met minimum quality standards and contextual factors using propensity score matching to assess SMC impact in Nigeria. In the intervention areas, six Local Government Areas (LGAs) each were selected from two states. Control LGAs in a third state were matched 1:1 with intervention LGAs on mean annual LGA-level temperature, rainfall and elevation, using the nearest neighbor method without replacement and with a caliper width of 0.8. Health facility register data were abstracted from 133 public facilities across the LGAs in the three states covering 2017–2019. A Wilcoxon signed rank test for matched pairs was conducted to assess impact of SMC implementation on monthly malaria incidence before (March-June) and during the SMC season (July-October). Incidence was measured as monthly confirmed case counts among the 0-59 months population in each season. Preliminary results indicate incidence was statistically significantly higher in one intervention area than the control area before the SMC season (z=-6.970, p<0.001) and during SMC (z=-5.651, p<0.001). Incidence in the second intervention area was statistically significantly higher than the control area before the SMC season (z = 2.682, p<0.001); no significant difference was noted during the SMC season (z=0.297, p=0.769). Further analyses will include 5-10 year olds as a comparator group and adjusting for confounding factors such as seasonality, other interventions, baseline incidence. Additional methods for selecting control groups and analyses with controls will be explored to include adjustments beyond seasonal factors

1226

BEDNET NON-ADHERENCE IN A LOW TRANSMISSION SETTING FOLLOWING 5 YEARS OF SUSTAINED IRS IN UGANDA

Paul Joseph Krezanoski[1], Grant Dorsey[1], John Rek[2]

[1]*University of California, San Francisco, San Francisco, CA, United States,* [2]*Infectious Disease Research Collaborative, Kampala, Uganda*

Combining indoor residual spraying (IRS) with insecticide treated bednets (LLINs) can achieve significant reductions in malaria transmission. LLINs remain the mainstay of malaria prevention, so it is important to understand how decreases in malaria transmission after 5 years of sustained IRS affect the use of LLINs. Eighty households and 526 individuals in Nagongera, Uganda were enrolled in a longitudinal cohort were followed from October 2017 - 2019, with biweekly mosquito collections and biweekly reported bednet use for every individual. Multi-level mixed logistic regression was utilized to estimate associations between age, mosquito exposure and household wealth on the odds of non-adherence. An age-matched case-control design, analyzed with conditional logistic regression, was utilized to estimate the association between non-adherence over various time windows and the odds of malaria. LLIN use was generally high in the first 18 months and then there was a significant decline in use in the final six months of the study. Children used bednets less than adults throughout the two years and, in the final six months, non-adherence compared to adults was 3.31 [95% CI: 2.30 to 4.75; p<0.001] for children under 5 years and 6.88 [95% CI: 5.01 to 9.45; p<0.001] for children 5 to 17 years. Non-adherence was also associated with the presence of fewer mosquitoes in the first 18 months (3.25 [95% CI: 2.92 to 3.63; p<0.001]) and with lower wealth in the final six months (OR: 5.09 [95% CI: 1.17 to 22.2; p=0.03]). Twenty-two cases were included in the case-control study. Any reported non-adherence over 8 weeks associated with 15.0 (95% CI: 1.95 o 114.9; p=0.009) the odds of malaria. In summary, as malaria transmission declined in this setting, LLIN use declined as well. Despite knowing the importance of LLIN use by children, adults used LLINs more than children and non-adherence was associated with higher odds of malaria. We conclude that reinvigorated behavior change programs and other strategies for sustaining high levels of LLIN use and ensuring coverage of vulnerable populations are needed as malaria transmission patterns change with the successful use of IRS.

1227

STRATIFICATION OF MALARIA AT-RISK VILLAGES IN CAMBODIA

Pengby Ngor

Mahidol-Oxford Tropical Medicine Research Unit (MORU), Bangkok, Thailand

Malaria stratification is used to guide distribution of limited resources and target interventions for malaria elimination. Prior to 2012, malaria risk stratification of villages in Cambodia was based on the distance from the forest using maps created in 1996 and which included many forested areas that have since been cleared. This stratification has since been updated using the local knowledge of senior staff at the National Malaria Control Programme (CNM) and in the provinces but a more robust methodology was needed. In 2019, CNM have been using individual case data from village malaria workers (VMWs) and health facilities to re-stratify their at-risk villages. The stratification process involves combining updated forest maps derived from satellite images, individual case data collected by VMWs and health facilities, distance from the villages to the nearest health facility and updated population counts. Village level incidence rates and forest cover by village were calculated, as were the village level incidence rates for the whole country. These were then combined and used to produce an easily updatable risk metric for each village using a specially created scoring system classifying villages into six levels of risk from none to very high. As individual cases data are uploaded to the Malaria Information System (MIS) via smart phones and tablets of VMWs and health center staff in real time, this allows the stratification to be kept up to date by CNM to guide ongoing planning as the malaria situation evolves. The risk categories are used to deployment of different intervention packages to help optimize efficient use of resources and accelerate progress towards the malaria elimination goal.

1228

A HIGH-THROUGHPUT, RAPID STRATEGY FOR ACTIVE SCREENING & ELIMINATION OF MALARIA IN GREATER MEKONG SUBREGION

Hongru Pian, Mingzhu Yang, Zhi Zheng

Institute of basic medicine, Chinese academy of medical sciences, Beijing, China

In regions of low malaria tansmission, sensitive and reliable detection technology are needed to reduce transmission and achieve elimination. Capture and Ligation Probe-PCR(CLIP-PCR) has been demonstrated as a high throughput RNA quantification technology for sensitive identification of malaria. In this study, we sought to adopt CLIP-PCR in large-scale malaria screening to reduce transmission effiiently. CLIP-PCR can detect *Plasmodium falciparum* diluted in whole blood lysate at a concentration as low as 0.001parasite/ul. When PCR coupled with a matrix pooling strategy, the assay can increase throughput to thousands of samples per day while reducing the asssay cost low to $0.7 for each sample. We applied the method to an active screening in Laza city in the second special administrative region of Kachin State of Myanmar in 2019 and 2020. Experimental group and control group were set to determine the effectiveness of CLIP-PCR in malaria elimination. In experimental group, about 1000 participants were repeatedly screened every two months throughout the year with CLIP-PCR and all positive individuals were treated. In control group, about 1000 participants were screened thrid times throughout the year and only RDT positive ones were treated. The prevalence of CLIP-PCR positivity was compared between the two groups at baseline and during follow-up. In both group, participants were similar on demographic characteristics and prevalence of CLIP-PCR positivity at baseline. After baseline, the prevalence of CLIP-PCR positivity in experimental group was dropped down from 1.3% to 0.65%. Meanwhile, the prevalence of CLIP-PCR positivity in experimental group was stay the same. In this study, CLIP-PCR show high-sensitivity and high-thoughput with low cost and labor for malaria screening. Active case detection, consisting of active screening with CLIP-PCR coupled with a matrix pooling strategy and treatment of infected individuals, can efficiently reduce malaria transmission. And this strategy could contribute to the elimination of malaria in regions of low transmission.

1229

COMMUNITIES' KNOWLEDGE, ATTITUDE AND PRACTICES TOWARDS MALARIA IN THE CONTEXT OF INTRODUCING MULTIPLE FIRST LINE THERAPIES IN THE HEALTH DISTRICT OF KAYA, BURKINA FASO: A MIXED-METHODS STUDY

Jean Moise T. Kabore[1], Mohamadou Siribie[1], Denise Hien[1], Issiaka Soulama[1], Seydou Sombie[1], Nouhoun Barry[1], Alfred B. Tiono[1], Christian Burri[2], Andre Marie Tchouatieu[3], Sodiomon B. Sirima[1]

[1]*Groupe de Recherche Action en Santé (GRAS), Ouagadougou, Burkina Faso,* [2]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [3]*Medicine for Malaria Venture (MMV), Geneva, Switzerland*

Multiple first line therapies (MFTs) for the management of uncomplicated malaria is a promising and innovative strategy to extend the therapeutic life of the artemisinin combinations therapies (ACTs) by reducing drug pressure and slowing the spread of resistance without putting lives at risk. As a prelude to the implementation of a pilot MFT program, we carried out from Nov 2018 to Jan 2019, a cross sectional study with mixed-methods design, to assess community perception about malaria and the factors influencing treatment seeking behavior toward malaria in the health district of Kaya. Quantitative data were collected in 1,148 sample households through face-to face interviews using a semi-structured questionnaire. Qualitative exploration was done in 17 focus group discussions (FGD) including 8 to 10 participants each. Study participants included heads of households, mothers/caregivers of children, adult participants. During the household survey, 1,400 participants have been interviewed; 72.9% preferred seeking treatment from health centers as a first recourse of care, 5.7% treated at home with the remaining drug stock and only 0.4% preferred traditional healers. The main reason of visiting health center is the proximity (OR=1.8, CI [1.2; 2.7]) or good reputation (OR=27.4 CI [10.7; 70.1]). 66.5% of those treated, started the treatment with 24 hours after the onset of presumed malaria symptoms but only 67.1% have taken their ACT treatment for 3 days. Geographical proximity to health center, good reputation of providers, availability of drugs and previous personal experience significantly affect treatment-seeking behavior. During FGDs, most of respondents had a good knowledge on malaria symptoms, prevention tools and effective treatment. The main reasons for participants to seek care were: behavioral change regarding malaria treatment, belief that malaria is hospital treated disease and free medication for children under five. The good level of community knowledge on malaria, coupled with community behavior in seeking care at health centers, could be a potential facilitator for the implementation of the MFT pilot program.

1230

PREVALENCE AND RISK FACTORS OF ASYMPTOMATIC *PLASMODIUM FALCIPARUM* MALARIA INFECTIONS IN CHILDREN BELOW FIVE YEARS IN SEASONAL MALARIA CHEMOPREVENTION SETTINGS IN NORTHERN CAMEROON AND SOUTH SENEGAL

Innocent M. Ali[1], Isaac Akhenaton Manga[2], Valery Pacome Tchuenkam[3], Akindeh Mbuh Nji[1], Jude Daiga Bigoga[4], Babacar Faye[5], Colin Sutherland[6], Cally Roper[6], Dorothy Fosah Achu[7], Wilfred Fon Mbacham[1]

[1]*Laboratory for Public Health Research Biotechnologies, University of Yaounde 1, Yaounde, Cameroon,* [2]*Faculty of Medicine, Pharmacy and Odontostomatology, Cheick Anta Diop University, Dakar, Senegal,* [3]*Department of Biochemistry, University of Dschang, Dschang, Cameroon,* [4]*Molecular Parasitology and Disease Vector Laboratory, The Biotechnology Centre, University of Yaounde I, Yaounde, Cameroon,* [5]*Department of Parasitology, Faculty of Medicine and Odontostomatology, Cheick Anta*

Diop University, Senegal, Dakar, Senegal, [6]London School of Hygiene & Tropical Medicine, London, United Kingdom, [7]National Malaria Control Program, Yaounde, Cameroon

Among the several strategies recommended for the fight against malaria, seasonal malaria chemoprevention (SMC) targets children 3 months to 5 years in Sahel regions of Africa to reduce mortality and morbidity. We set out in this study to compare the level of asymptomatic infections in areas of SMC compared with others without it in Cameroon and Senegal. We used a two staged cluster sampling approach to collect information and blood spots for rapid malaria testing from 5993 consenting household members in Cameroon [North (SMC) and Adamou (no SMC) and Senegal(Saraya and Kolda, all SMC)]. The primary outcome was asymptomatic malaria by RDT and by qPCR in children 3 months to 5 years. We summarised measures as frequency counts and logistic regression analysis for risk factors. We modelled significant risk factors A *p* value=0.05 was threshold of significance. The R package was used for analysis. Together, 2059/2300 in North and 2234/2300 in Adamaoua took part in the survey in Cameroon. The overall prevalence of asymptomatic malaria in children 3 months to 5 years by RDT and qPCR was 36.5% (95% (33,3-39,8) by RDT and 18.3%(95% (16.6-19.9) by qPCR in the North and 37,3(34,9-39,8) by RDT and 14.9%(95% (13.4-16.4) by qPCR in Adamaoua (P less than 0.05). By Contrast in Senegal, there was a significant lower proportion of asymptomatic infections by RDT or qPCR in general population and among children 3 months to 5 years compared to those greater than 5 years [RDT: 21% (19,8%-23,6%); 16.2%(13,2-19,8) vs. 23.7%(21,4-26,1)], respectively. P=0.001) or to children of same age in North Cameroon. In all sites, bednet ownership and use, and previous diagnosis and treatment were protective against asymptomatic malaria. Our analysis reveals lower level of asymptomatic malaria in children 3 months to 5 years in SMC regions of Cameroon and this may be a positive effect of SMC. Lower asymptomatic malaria in general population in Adamaoua was unexpected. Asymptomatic malaria is less prevalent in Senegalese children less than 5 years compared to children in North Cameroon which recently adopted SMC. Further studies are required to confirm these findings in other settings.

1231

DEVELOPMENT OF MALARIA FREEDOM FROM INFECTION SURVEILLANCE TOOLS TO INFORM NATIONAL AND SUBNATIONAL ELIMINATION STRATEGIES

Lindsey Wu[1], Luca Nelli[2], Adilson de Pina[3], Adam Bennett[4], Andrew Lover[5], Francois Rerolle[6], Valerie Scott[4], Henry Surendra[7], Riris A. Ahmad[8], Risalia R. Arisanti[9], Dyah A. Lesmanawati[10], Xuan Thang Nguyen[11], Ngo Duc Thang[11], Abdisalan Noor[12], Kim Lindblade[12], Gillian Stresman[1], Chris Drakeley[1]

[1]*London School of Hygiene & Tropical Medicine, Faculty of Infectious and Tropical Diseases, Department of Infection Biology, London, United Kingdom,* [2]*University of Glasgow, Institute of Biodiversity, Animal Health and Comparative Medicine, Glasgow, United Kingdom,* [3]*Ministério da Saúde Cabo Verde, Malaria Elimination Program (CCS-SIDA), Praia, Cape Verde,* [4]*University of California, San Francisco, Global Health Group, Malaria Elimination Initiative (MEI), San Francisco, CA, United States,* [5]*University of Massachusetts - Amherst, School of Public Health and Health Sciences, Department of Biostatistics and Epidemiology, Amherst, MA, United States,* [6]*University of California, San Francisco, Global Health Group, Malaria Elimination Initiative (MEI), San Francisco, CA, United States, San Francisco, CA, United States,* [7]*Eijkman-Oxford Clinical Research Unit, Eijkman Institute for Molecular Biology, Jakarta, Indonesia,* [8]*Universitas Gadjah Mada (UGM), Faculty of Medicine, Yogyakarta, Indonesia,* [9]*Centre for Tropical Medicine, Faculty of Medicine Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia,* [10]*Universitas Gadjah Mada (UGM), Yogyakarta, Indonesia,* [11]*National Institute for Malariology, Parasitology and Entomology, Ministry of Health Vietnam, Hanoi, Viet Nam,* [12]*WHO Global Malaria Programme, Geneva, Switzerland*

Surveillance forms the foundation of malaria elimination. However, a major challenge is determining the degree to which the absence of cases or infections reported in the health system provides evidence for elimination. Freedom from Infection (FFI) surveillance approaches have been used previously in veterinary epidemiology to estimate the probability that infection in a population is absent or below a given threshold. The Malaria FFI Project is partnering with a number of countries targeting national or subnational elimination to adapt these methods for malaria. Using monthly passive case detection data from Cape Verde, Northern Laos, Indonesia, and Vietnam, variations of FFI modelling approaches were used to estimate the probability of malaria elimination across a variety of epidemiological settings and elimination phases. This included nationwide data from 22 facilities in Cape Verde between 2014 and 2017, subnational data from 18 facilities in four districts of Northern Laos between 2013 and 2016, as well as preliminary datasets from Indonesia and Vietnam in 2016 and 2018, respectively. To further inform model parameters, district and health facility surveys were conducted in Indonesia and Vietnam to estimate key indicators of health systems surveillance capacity for elimination. This included data on malaria diagnostic and case management capacity, human resources and training, as well as case investigation and reporting practices. Preliminary results show notable differences in the probability of elimination across countries, districts, and health facilities. Sensitivity analysis indicates that differences are driven by variations in care seeking behaviour, facility testing rates, as well as duration and frequency of routine case reporting. These tools will be further refined to help identify components of the surveillance system that can be enhanced for more robust monitoring of elimination. Outcomes will also be used to design potential active surveillance strategies to supplement passive case detection and estimate time frames required to demonstrate elimination at national or subnational levels.

1232

EVALUATING THE COMMUNITY HEALTH WORKER NETWORK TO ACHIEVE AND SUSTAIN MALARIA ELIMINATION IN GRACIAS A DIOS, HONDURAS

Manuel Espinoza Garcia[1], Madeline Baird[1], Justin Lana[1], Kim Hanson[1], Sarah Park[1], Lizeth Cartagena[2], Ana Vallecillo[2]

[1]*Clinton Health Access Initiative (CHAI), Tegucigalpa, Honduras,* [2]*Secretary of Health (SESAL), Puerto Lempira, Honduras*

In Honduras, the extension of malaria diagnosis and treatment through its volunteer collaborator (CHW) network has been a key element of malaria elimination efforts for over fifty years. Since 2016, the department of Gracias a Dios has experienced an 89% decrease in malaria incidence. In response to shifting malaria epidemiology, Clinton Health Access Initiative (CHAI), in February 2020, supported a departmental Community Health Worker (CHW) program review to assess impact and identify strategies to ensure continued robust community surveillance. Quantitative analysis employed DHIS2 surveillance and program data to evaluate CHW contributions across selected malaria indicators from January 2017 to December 2019. Qualitative methods applied a framework, based on WHO guidance for CHW systems, in relation to six core components: (i) network management; (ii) training; (iii) supervision and supply chain; (iv) reporting and feedback; (v) health system linkage; (vi) service provision and community participation. Following implementation of strengthened CHW supervision, training and expanded use of rapid diagnostic tests (RDTs), case detection by CHWs increased fourfold, from 15% of cases in the first quarter of 2017 to over 66% over the same period in both 2018 and 2019. Despite an increase in community detection, patients delayed seeking CHW services, with 37.5% of cases diagnosed by CHWs more than a week following symptom onset in 2019. Interviews with ministry representatives (n=35) and community focus groups (n=5) contextualized this delay, describing patient resistance to testing, preference for self-medication and diminishing CHW motivation as malaria-only services

may become obsolete. CHWs and community members alike mentioned that an integrated package of CHW services would enhance patient treatment seeking and improve CHW ability to serve their community. This review identified areas for network strengthening, several of which were integrated into the country's current Global Fund application and demonstrates the ability to tailor WHO guidance into a useful framework for improving CHW networks in a malaria elimination context.

1233

IMPACT OF ENHANCED COMMUNITY CASE MANAGEMENT AND MONTHLY SCREENING AND TREATMENT ON THE HUMAN INFECTIOUS RESERVOIR FOR MALARIA

Katharine A. Collins[1], Alphonse Ouedraogo[2], Wamdaogo M. Guelbeogo[2], Shehu S. Awandu[1], Will Stone[3], Issiaka Soulama[2], Maurice S. Ouattara[2], Apollinaire Nombre[2], Amidou Diarra[2], John Bradley[4], Prashanth Selvaraj[5], Jaline Gerardin[6], Chris Drakeley[3], Teun Bousema[1], Alfred B. Tiono[2]

[1]*Department of Medical Microbiology, Radboud university medical center, Nijmegen, Netherlands,* [2]*Centre National de Recherche et de Formation sur le Paludisme,, Ouagadougou, Burkina Faso,* [3]*Department of Immunology and Infection, London School of Hygiene & Tropical Medicine, Faculty of Infectious and Tropical Diseases, London, United Kingdom,* [4]*MRC Tropical Epidemiology Group, London School of Hygiene & Tropical Medicine, London, United Kingdom,* [5]*Institute for Disease Modeling, Bellevue, WA, United States,* [6]*Department of Preventive Medicine, Northwestern University, Chicago, IL, United States*

Asymptomatic infections are a major obstacle in the path to malaria elimination. Many malaria-infected individuals in endemic areas do not experience symptoms that prompt treatment-seeking and can remain infectious to mosquitoes for many months. Reductions in malaria transmission could be achieved by detecting and treating these infections early with enhanced community case management (CCM) comprising active weekly screening for fever, and detection and treatment of infections in fever positive individuals using rapid diagnostic tests, or with monthly screening and treatment (MSAT). In a cluster-randomized trial we assessed the impact of these interventions on prevalence and transmissibility of malaria infections in an area of intense, highly seasonal malaria in Burkina Faso. 180 compounds with 907 subjects were randomized to: arm 1 - current standard of care with passively monitored malaria infections; arm 2 - standard of care plus enhanced CCM; or arm 3 - standard of care and enhanced CCM plus MSAT. The intensive 18 month follow-up (ending Feb 2020) involved 4 start/end season cross-sectional surveys and regular monitoring of parasitemia and gametocytemia in rolling surveys. Parasite prevalence by qPCR peaked at 89% in the transmission season (median 60,440 p/mL) and declined to 27% in the dry season (median 6,522 p/mL). A total of 997 clinical malaria episodes were passively detected; 108 mild clinical infections detected by CCM; and 629 infections detected by MSAT. 4.5% (35/771) of asymptomatic infections and 5.7% (7/123) of symptomatic infections were transmissible to mosquitoes in membrane feeding assays. Cross-sectional survey samples are still being examined molecularly; initial findings after 6 months of interventions suggest a measurable impact of CCM and MSAT on qPCR parasite prevalence (arm 1: 58% [95% CI: 52-63%] arm 2: 52% [95% CI: 46-58%]; arm 3: 44% [95% CI:38-50%]). If this initial observation is maintained this study could pave the way for a simple yet effective method to control malaria burden in areas of high transmission by abrogating infections before severe morbidity and development of transmissible gametocytemia

1234

THE ROLE OF MOBILE PAYMENT MODALITIES IN THE VILLAGE MALARIA WORKER PROGRAM IN CAMBODIA

Po Ly[1], Huy Rekol[1], Bou Khengthavrine[1], Siv Sovannaroth[1], Muor David[1], Neang Vanrith[1], Noun Sophear[1], Kimhong Gove[2], Michelle Pahl[2], Vichka Khy[2], Thary Cheav[2], Joshua Christenson[2], Chao Chi Chuong[3], Kimheang Ung[3]

[1]*National Center for Parasitology, Entomology, and Malaria Control, Phnom Pehn, Cambodia,* [2]*Clinton Health Access Initiative, Phnom Pehn, Cambodia,* [3]*United Nations Office for Project Services, Phnom Pehn, Cambodia*

Launched in 2004, the Village Malaria Worker (VMW) network plays an essential role in extending malaria testing and treatment service to those living in high endemic and hard to reach communities in Cambodia. In 2016, The Global Fund to Fight AIDS, Tuberculosis and Malaria, which funds malaria activities in Cambodia, issued the immediate implementation of program management guidelines to increase grant transparency and accountability. This included the requirement that all financial transactions be processed via electronic payments. In the absence of a system that could replace cash payments in remote VMW catchment areas, this transition resulted in the deactivation of the VMW network from 2016 to 2017. VMWs receive monthly payments, and timely disbursement is imperative to performance. The discontinuation of routine payments had a detrimental impact on VMW retention and malaria elimination activities in Cambodia. This resulted in a sharp drop in testing and treatment, with 15,365 tests conducted and 5,041 identified cases by VMWs in 2016, the lowest recorded level since the implementation of the VMW network. In response, the national malaria program launched an electronic payments model in 2018 to accommodate a majority of VMWs and comply with requirements. Three modalities were selected: Wing/E-money online Portal Service, account-to-account transfers, and electronic payment from outlets to VMW mobile phones. Monthly meetings in December 2019 reported over 80% of VMWs have received payment on time. The majority of VMWs receive funds through Wing accounts (64%), with e-Money (24%) and True Money (1%) providing additional electronic payment options. The remaining 11% of VMWs receive payments in cash and plan to transition to Wing in 2020. As a result, VMWs are continuing their substantial contribution to malaria case management, conducting 426,165 (70% of public sector) malaria tests and treating 17,194 (53% of public sector) confirmed malaria cases in 2019. This mobile payment model can be adopted by other programs to increase transparency and accountability while minimizing transaction costs and supporting volunteer retention.

1235

DATA QUALITY OF MALARIA INCIDENCE INDICATORS IN THE DEMOCRATIC REPUBLIC OF THE CONGO

Christian Burri[1], John Kamwina[1], Giulia Delvento[1], Solange Umesumbu[2], Didier Kalemwa[3], Eric Mukomena[2]

[1]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [2]*Programme National de Lutte contre le Paludisme, Kinshasa, Democratic Republic of the Congo,* [3]*Swiss Tropical and Public Health Institute, Kinshasa, Democratic Republic of the Congo*

In 2017, 245 million Rapid Diagnostic Tests (mRDTs) were distributed by National Malaria Control Programs (NMCPs). In the DRC, malaria is identified as one of the three leading causes of death in children under five years. The NMCP recommends that all suspected cases of malaria be tested before being treated. Since 4 years, malaria surveillance in the DRC relies on data collected through the web-based District Health Information System 2 (DHIS2). In addition, routine data are fed into a malaria specific NMCP Excel database. No malaria indicator surveys have been conducted in the DRC, Demographic and Health Survey Multiple Indicator Cluster Surveys in 2013 and 2017, respectively. With support from partners, the PNLP established malaria sentinel sites in all 26 provinces, which report on active community based studies and passive routinely collected health facility records - unfortunately, funding of this initiative ceased in 2018.Like

in other countries, concern exist about data validity, representativeness and completeness of the system. The utilization and performance of mRDTs are still suboptimal and many challenges, limitations and operational shortcomings are not fully understood. We therefore carried out a review of routine malaria data from 2015-2019 to assess the data quality at the NMCP in the DRC and to identify bottlenecks. The main issues identified included:- mRDT positivity over time was relatively stable at about 70%- The positivity rate of routine data is significantly higher compared to the Multiple Indicator Cluster Survey (MICS-Palu, DRC, 2017-2018): Prevalence was 38.5% by mRDT, 30.9% by microscopy- There are considerable fluctuation of total number of mRDT performed per year. It is not possible to attribute this to e.g. stockouts since these data are largely missing- The ratio of patients receiving ACTs among those with a positive mRDT increased by over 50% from 2015 to 2016. This coincides with the year when the PNLP included the goal of treating over 80% of malaria positives with an ACTA mixed-method, cross-sectional study to triangulate the results of the data review will be conducted in selected health Zones to as a next step.

1236

USING SPATIAL INTELLIGENCE TO INFORM MALARIA SOCIAL BEHAVIOR CHANGE IN ZAMBIA

Todd Jennings[1], Derek Pollard[2], Anna Winters[2], Frazer Bwalya[2], Christina Riley[2]

[1]*PATH Malaria Control and Elimination Partnership in Africa (MACEPA), Lusaka, Zambia,* [2]*Akros, Lusaka, Zambia*

Zambia's progress toward malaria elimination is grounded in the capture and use of quality and timely data from many sources including epidemiology, intervention coverage, entomology, commodity supply, and rainfall to inform decision-making and program action. The country is now applying a digital 'spatial intelligence' platform (Reveal) to map communities down to household level and improve intervention targeting. The malaria program is seeking to apply the same precision to social behavior change (SBC) data capture and delivery. During a 2018/2019 mass drug administration (MDA) campaign, SBC questions were added to a household survey in three districts in Southern Province. Conducted using a spatial intelligence platform designed to provide effective delivery of health interventions, the survey aimed to better understand malaria knowledge, message penetration, and preferred communication channels. Households of each focus community were mapped using satellite imagery to learn where people live, and to navigate field teams to households to ensure houses were reached and implementation was efficient. The survey response data were overlaid with epidemiological data—malaria case numbers by health facility catchment area—to help prioritize any malaria messaging response activities to the community health worker zones reporting higher malaria cases. SBC activities were then deployed using Reveal to cover houses within areas found to have gaps in malaria knowledge, thus improving SBC coverage over time. Application of this platform for SBC (a first for Zambia) has shown potential to not only add to the country's evidence base, but also to provide a data-driven approach to efficiently target appropriate SBC activities. By identifying gaps—areas with limited access to the usual communication channels; where people are not at home due to seasonal migration for fishing or farming; areas of low malaria knowledge or high refusals—SBC delivery and intervention uptake can be improved. It would be useful to evaluate this targeted and tailored approach to assess program impact.

1238

EFFECTIVE COMMUNITY-BASED MALARIA CASE MANAGEMENT THROUGH THE INTERVENTION OF COMMUNITY HEALTH AGENTS (ADECOS) IN ANGOLA

Alfredo Francisco

World Vision, International, Luanda, Angola

Angola, just as other African countries, still have gaps in the national health systems, hence there is need in the strengthening of data collection and use for improved evidence-based decision-making. Despite continuous efforts by government, critical constraints such as timely and reliable data collection, analysis are in vital need for providing accurate information for decision makers, so as to provide timely epidemiological control and management at community, especially in very remote areas. Government through Public Health, with the partnership of World Vision; funded by Global fund; and other implementing partners like USAID, E8; considered using ADECOS in selective primary care proposal (malaria), creating increasing health service demands. ADECOS being a family health team corresponds to one of the care points in the municipal health system network. ADECOS live in the community itself, have a more social than technical profile, with full-time availability to perform their activities. Among other tasks, they work with families within a defined geographical base (micro area) and register and follow these families. The ADECOS are responsible for analysing community needs; being active in health promotion and disease prevention actions, especially involving children, women, adolescents, elderly and physically and mentally-impaired persons; participating in basic hygiene and environmental improvement actions; participating in health team meetings and other health events with the community. Categorically, the ADECOS represent the link between the professional team and the community, with the role of *translating* the scientific to the popular universe, of facilitating people's access to health services. Despite difficulties, the benefits the ADECOS work all over the country have brought to the Angolan population's health are undeniable, with decreased mortality and morbidity levels and improved rates in some health actions, accompanied by the community's valuation of their work. This approach is very sustainable, with regards to scale-up primary health services across the country, especially in most remote areas.

1239

ANTIMALARIAL PROPHYLAXIS FOR FOREST GOERS IN CAMBODIA: STRATEGIES FOR IMPLEMENTATION AND THEIR IMPLICATIONS

James J. Callery[1], Rupam Tripura[1], Thomas J. Peto[1], Chhoeun Heng[1], Mom Ean[1], Lorenz von Seidlein[1], Franca Conradis-Jansen[2], Monnaphat Jongdeepaisal[1], Panarasri Khonputsa[1], Chris Pell[3], Worarat Khuenpetch[1], Siv Sovannaroth[4], Richard J. Maude[1]

[1]*Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand,* [2]*Medizinische Fakultät, University of Heidelberg, Heidelberg, Germany,* [3]*Amsterdam Institute for Social Science Research, Amsterdam, Netherlands,* [4]*National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia*

In the Greater Mekong Subregion, the remaining pockets of malaria transmission are increasingly confined to remote forested areas. Livelihood activities in forest-fringe villages often revolve around subsistence agriculture and forest going - high-risk activities for malaria. Antimalarial prophylaxis could be an appropriate strategy to protect these high-risk, hard-to-reach individuals, but implementing this novel approach presents many practical and cultural challenges. A study implementation strategy was developed in collaboration with the local community prior to beginning an antimalarial prophylaxis trial involving forest goers in Stung Treng, Cambodia. As most forest work is illegal, one major issue to overcome was the building of trust. Stakeholders, such as local authorities, community health workers and forest goers participated in developing an engagement strategy to identify, sensitise and mobilise this ethnically diverse hard-to-reach group. A number of strategies were deployed following initial stakeholder engagement: (1) recruiting known forest goers (previously involved in a malaria treatment trial) to facilitate the contacting of other forest goers; (2) building community trust through school engagement activities; (3) learning and understanding the local terminology and forest going routines, to develop communication tools suitable for non-Khmer speaking and low-literacy groups; (4) involving trusted community members in village enrolment meetings; and, (5) regularly collecting feedback from study participants upon study recruitment, including the digital capture of forest experiences. The implementation strategy has continued to adapt in response to new and developing challenges, such as the enforcement of bans on forest travel.

At the time of abstract submission, the prophylaxis trial was ongoing. We will report our preliminary findings and discuss their implications for malaria elimination strategies involving remote forested communities.

1240

GENETIC EPIDEMIOLOGY REVEALS INFECTION CONNECTIVITY AND INCIDENCE CORRESPONDENCE AMONG SÉNÉGAL MALARIA INFECTIONS IN 2019

Daouda Ndiaye[1], Rachel F. Daniels[2], Yaye Die Ndiaye[1], Stephen F. Schaffner[3], Katherine Figueroa[3], Angela M. Early[3], Claudia R. Taccheri[2], Aida S. Badiane[1], Awa B. Deme[4], Fatou Ba Fall[5], Medoune Diop[5], Alioune Badara Gueye[5], Ibrahima Diallo[5], Moustapha Cisse[5], Doudou Sene[5], Daniel L. Hartl[6], Bronwyn L. MacInnis[7], Dyann F. Wirth[2], Sarah K. Volkman[2]

[1]*Cheikh Anta Diop University, Dakar, Senegal,* [2]*Harvard T.H. Chan School of Public Health, Boston, MA, United States,* [3]*Broad Institute, Cambridge, MA, United States,* [4]*Dantec Teaching and Research Hospital, Dakar, Senegal,* [5]*Senegal National Malaria Control Program, Dakar, Senegal,* [6]*Harvard University, Cambridge, MA, United States,* [7]*Broad Institute, Boston, MA, United States*

Genetic analysis was used to detect the connectivity between Sénégal *Plasmodium falciparum* infections from the 2019 transmission season across high burden and pre-elimination settings. We hypothesized that parasite genetic relatedness reflects transmission and could inform intervention targeting and impact evaluation. We genotyped 1,356 and sequenced 628 malaria samples from 26 different health-posts representing distinct transmission settings (incidence: <1/1000 to >450/1000). Health posts included sentinel sites and settings where campaign-level interventions were ongoing or planned. Genotyping data of 24 single-nucleotide polymorphisms were analyzed for relatedness using identity by state (IBS) and whole genome sequencing for relatedness using identity by descent (IBD). Only samples with pairwise IBS or IBD of > 0.95 were included. Overall, 16% (211/1,356) of sampled parasites shared genotypes, forming 60 different IBS-clusters. Almost all parasites in clusters (97%, 204/211) were within a health-post; while half (47%, 99/211) included parasites across health-posts. Patterns included Diourbel, a site of moderate incidence, exhibiting large IBS-clusters containing 77% of the parasites, indicating significant local transmission with clonal expansion. Thiès, a low incidence site and major travel hub, comprised 6% (78/1,356) of parasites sampled and 16% (10/60) of IBS-clusters, with most clusters containing a parasite from another health-post, indicating significant gene flow across geography. To evaluate the relationship between genetic metrics and epidemiological indicators, we compared health-post incidence with a genetic score calculated by combining the fraction of the parasite population sampled that was identical with that which was monogenomic. We found a Pearson correlation of -0.65 between incidence and genetic score. These data indicate a correspondence between genetic and epidemiological indicators, and reveals otherwise undetected patterns of relatedness among infections that can gauge transmission and guide appropriate intervention targeting and selection.

1241

ASSESSMENT OF INSECTICIDE TREATED NETS USE AFTER THE 2018 MASS DISTRIBUTION CAMPAIGN IN DANGASSA, MALI

Drissa Konate, Sory I. Diawara, Bourama Keita, Aboubacar Fomba, Nafomon Sogoba, Sekou F. Traoré, Seydou Doumbia, Mahamadou Diakite

MRTC/USTTB, Bamako, Mali

Insecticide-Treated Nets (ITNs) use remains one of major strategies in malaria control intervention in Mali. The Mass Distribution Campaign (MDC) is considered as a best approach than others (children under one-year immunization and Antenatal care during pregnancy) to obtain high coverage rate in terms of accessibility. The goal of this study was to assess the use of ITNs in Dangassa, 7 months after the last MDC. A cross-section survey was conducted in Dangassa village, located at 4 km from river Niger, in December 2018. The data were collected on 177 households selected from which 868 ITNs were identified. The logistic regression was used to determine the factors associated with ITNs use. The coverage of ITNs during the last MDC was 97.2% in Dangassa. Among the ITNs hanging in bedrooms, 63.3% were used to sleep the last night. Using logistic regression, household size, passable condition of ITNs (OR=2.64, p=0.001) or bad condition of ITNs (OR=2.23, p=0.009) were associated with non-use of ITNs. The high number of ITNs available in household was significantly associated with their use (p=0.049 & p=0.002). The MDC remains a better approach to ensuring the accessibility of ITNs. The high number of ITNs and their good condition are significantly associated positively with the ITNs use.

1242

GENETIC DIVERSITY AND THE REPRODUCTION OF *PLASMODIUM VIVAX* IN RIVERINE VILLAGES ALLOCATED TO DIFFERENT TEST-AND-TREAT INTERVENTIONS IN THE PERUVIAN AMAZON

Roberson Ramirez[1], Paulo Manrique[2], Angel Rosas-Aguirre[3], Juan Contreras-Mancilla[1], Jose Barboza[1], Niko Speybroeck[3], Alejandro Llanos-Cuentas[2], Joseph Vinetz[4], Dionicia Gamboa[1]

[1]*Laboratorio ICEMR-Amazonía, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru,* [2]*Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru,* [3]*Research Institute of Health and Society (IRSS), Université catholique de Louvain, Brussels, Belgium,* [4]*Section of Infectious Diseases, Department of Internal Medicine, Yale School of Medicine, New Haven, CT, United States*

Knowledge on parasite diversity, transmission dynamics, and mechanisms of adaptation to environmental and interventional pressures can contribute to the design/adjustment of control strategies. This study prospectively assessed the genetic diversity metrics and the reproduction of *Plasmodium vivax*in fifteen endemic riverine villages in Mazan district, Loreto department between March 2016 and September 2017. In addition to the routine passive case detection, study villages were allocated to have three population-wide test-and-treat interventions before the high malaria transmission season starting in March 2016 (month M0). Interventions 1 and 2 had respectively one (M0, M12) and three (M0, M1, M2, M12, M13, M14) yearly population screenings by microscopy. Intervention 3 had two (M0, M1, M12, M13) yearly population screenings by microscopy and PCR. All confirmed infections were treated. Using sixteen microsatellite markers, we genotyped *P. vivax* parasites found in 92 samples collected in M0, M12 and M18. Overall, moderate to high population differentiation with low genetic flow (Fst> 1.3) was found along the study period, regardless the intervention implemented in villages. A significant decrease ($p<0.05$) in genetically related haplotypes was only observed in villages with the first intervention. Overall and by intervention group, the genetic population metrics did not change over time, and bottlenecks were not found. The analysis at micro-geographic scale (including parasite genotyping data previously collected) showed a reduction in the number haplotypes in Libertad village (where intervention 3 was implemented) in comparison to 2015. The stability in genetic diversity metrics despite interventions may evidence the vulnerability of villages to the entry of genetically unrelated haplotypes and consequently a permanent threat to malaria resurgence in villages. Further analyses at village level and their integration with the temporal and geographic evolution of parasitological and entomological indicators will provide further insights about this threat.

1243

A MOLECULAR LABORATORY IN THE FIELD. KEY TO SUCCESS FOR MALARIA TRANSMISSION AND ELIMINATION SURVEYS

Haddy Nyang, Abdullahi Ahmad, Natalie Hofmann, Lamin Camara, Blessed Etoketim, Sainabou Drammeh, Umberto D'Allesandro, Mamadou O. Ndiath

Medical Research Council, Fajara, Gambia

In regions with low malaria endemicity, asymptomatic infections pose a great challenge to elimination as they provide a reservoir of infection for continuous transmission. Accurately detecting asymptomatic infections, which are often characterised by low parasite density, require highly sensitive molecular diagnostics tools. These are often not available in remote field settings thus complicating studies investigating the true extent and transmission potential of asymptomatic infections. In the Upper River Region of The Gambia, where malaria prevalence remains moderately high, we set up a molecular laboratory in the field to support a study investigating the relationship between parasite density and transmission success in asymptomatic infections. A new molecular laboratory was set up in the MRC Basse field station to run a quantitative varATS PCR assay with a limit of detection of 0.1 parasites/µl and a maximum turnaround time of 48 hours. The 48hr turnaround time allowed timely sample collection from study participants with ≥0.1 parasites/µl for direct membrane feeding assay (DMFA). Prior to analysing patient samples, we validated the varATS qPCR assay to ensure sensitivity, specificity and no cross contamination of the assay in the field. Our new assay in the field had a limit of detection of 0.02-0.1 parasites/µl of blood, which corresponds to the detection limit of other established molecular labs. We successfully processed 3,297 samples across 2 cross sectional surveys, with a daily average of 60 samples. All samples were processed within the target turnaround time of 48 hours. 278/3297 (8.4%) samples were PCR positive, of whom 103 (37%) underwent DMFA. The set-up of a molecular laboratory in the field minimized fluctuation of parasite density between testing and DMFA, thereby ensuring that the true infectivity is measured.

1244

THE IMPACT AND LIMITATIONS OF REACTIVE CASE DETECTION UNDER REALISTIC PROGRAMMATIC CONSTRAINTS

Joshua G. Suresh[1], Marita Zimmermann[1], Gillian Stresman[2], Jackie Cook[2], Marie-Reine Rutagwera[3], John Miller[3], Busiku Haimanza[4], Caitlin Bever[1]

[1]*Institute for Disease Modeling, Bellevue, WA, United States,* [2]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [3]*PATH, Lusaka, Zambia,* [4]*Zambia NMEC, Lusaka, Zambia*

Reactive Case Detection (RCD) is an intervention which aims to target spatially distinct pockets of transmission through small followup campaigns in and around the households of individuals who seek treatment. Despite the appealing simplicity of RCD, the impact and cost-effectiveness of this intervention is unclear. Modeling studies thus far have often assumed idealistic conditions such as an unlimited number of followups per month or that every index case will be followed up to a radius of 140 meters around the index household. Our aim in this work is to study the impact RCD under more realistic programmatic capacity constraints using a spatial model of transmission. To estimate a realistic level of RCD capacity, we use the Zambia DHIS2 data on community health-workers (CHWs) performing RCD country-wide from 2017-2019. These volunteer CHWs perform up to ~5 RCD followups per month, per their training, and typically test and treat up to 20 people per followup. We find that RCD has the greatest impact when CHWs are able to follow up most or all of the passive cases that present each month; RCD is less effective at higher levels of transmission, where CHWs become overloaded and are unable to follow up all the passive cases they encounter. When RCD is performed at the level observed in the DHIS2 data, the intervention decreases transmission by roughly 40% when added in settings where prevalence is less than 10%, or about 200 cases per 1000 per year; this range aligns well with Zambia's current stratification strategy. We find that doing the RCD followup sweep as a test-and-treat versus a focal mass drug administration MDA have similar impacts on transmission, except at higher transmission intensities (where RCD is unlikely to be utilized anyways). The model suggests that adding RCD is comparable to increasing health-seeking rates by 20% in under-5's; this latter alternative might be achieved by increasing the number of CHWs in the country or with an active screening approach. The cost-effectiveness of these various near-elimination strategies is compared, as well as probability of and time to elimination, for a range of plausible importation levels.

1245

COBALT PORPHYRIN-PHOSPHOLIPID LIPOSOMES FOR SPONTANEOUS NANOLIPOSOMAL ANTIGEN PARTICLEIZATION AND ENHANCED IMMUNOGENICITY

Jonathan Lovell

SUNY Buffalo, Buffalo, NY, United States

Porphyrin-phospholipid (PoP) stably incorporates in liposome bilayers [1]. Cobalt can be chelated to generate CoPoP, which, when incorporated into bilayers results in stable functionalized with his-tagged antigens with simple incubation [2]. We recently reported that this technology is effective with malaria transmission blocking antigen, Pfs25 [3]. Induced IgG levels were higher than conventional adjuvants and a durable antibody response was observed. CoPoP liposomes have potential for applications in transmission blocking vaccination research, where high antibody generation and capacity for multiplexing, features that are useful for transmission blocking [4].One newly uncovered mechanism of enhanced immunity that appears to be associated with enhanced antigen uptake of the spontaneously-induced particles by various antigen-presenting cells in draining lymph nodes. Additional mechanisms include induction of antigen presentation machinery within phagosomes, and activation of dendritic cells.The CoPoP approach offers seamless antigen multiplexing capability. Spontaneous, biostable his-tagged antigen binding is observed for a wide range of antigens. We have shown up to 10 his-tagged peptides or 5 his-tagged proteins can simultaneously bind CoPoP liposomes and induce specific antibodies in immunized mice.Taken together, CoPoP liposomes appear to be promising next-generation vaccine adjuvant and delivery system for a variety of antigens in malaria and other infectious diseases.

1246

CO-DELIVERY OF MULTISTAGE MALARIA ANTIGEN USING COPOP LIPOSOEMES AS A POTENT VACCINE

Wei-Chiao Huang[1], Moustafa T. Mabrouk[1], Bingbing Deng[2], Carole A. Long[2], Kazutoyo Miura[2], Yimin Wu[3], Jonathan F. Lovell[1]

[1]*University at Buffalo, Amherst, NY, United States,* [2]*National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States,* [3]*PATH's Malaria Vaccine Initiative (MVI), Washington, DC, United States*

The circumsporozoite protein (CSP) is a major target for pre-erythrocytic malaria vaccine development, which could generate antibodies to protect against infection to host. Pfs230, a *Plasmodium falciparum* sexual-stage surface protein, is a candidate for malaria transmission-blocking vaccine (TBV) development. In this study, CSP and Pfs230 are combined to enhance both protection and transmission-reducing activity. Liposomes containing cobalt-porphyrin phospholipid (CoPoP) and synthetic lipid adjuvant monophosphoryl lipid A (MPLA) have the ability to facilitate particleization of His-tagged antigen. In this study, His-tagged recombinant CSP and Pfs230D1 were mixed with CoPoP liposomes to form dual-antigen vaccine. Fluorophore-labeled CSP and fluorophore-labeled Pfs230D1+ were used to detect binding kinetics and stability. Mice immunized with CoPoP liposomes admixed with Pfs230D1+ elicit strong IgG titer with transmission-reducing activity compared to mice immunized with Alum. Similar results were observed with mice immunized with dual-antigen formulated with CoPoP liposomes, and mice could generate antibodies with strong transmission-blocking activity. Antibodies induced

by combination of CSP and Pfs230D1+ admixed could also recognize sporozites and gametocytes. On the other hand, dual-antigen admixed with Alum shows weak specific antibody generation. We also immunized C57BL/6J mice and measured intracellular cytokines in CD4 T cells and CD8 T cells. The data shows that dual-antigen formulated with CoPoP liposomes with QS21 could generate higher population of specific CD4 T cells and CD8 T cells. Taken together, CoPoP liposome could serve as an adjuvant platform to deliver dual-malaria antigens to target different stage of parasite.

1247

MULTISTAGE VACCINE CANDIDATES INDUCE ANTIBODIES THAT BLOCK PRE-ERYTHROCYTIC DEVELOPMENT AND TRANSMISSION OF *PLASMODIUM FALCIPARUM*

Matthijs M. Jore[1], Susheel K. Singh[2], Bishwanath K. Chourasia[2], Amanda Fabra-García[1], Roel Heutink[1], Marga van de Vegte-Bolmer[1], Geert-Jan van Gemert[1], Michael Theisen[2]

[1]*Department of Medical Microbiology, Radboud university medical center, Nijmegen, Netherlands,* [2]*Centre for Medical Parasitology at Department of International Health, Immunology and Microbiology, University of Copenhagen & Department for Congenital Disorders, Statens Serum Institut, Copenhagen, Denmark*

Malaria continues to be one of the deadliest infectious diseases world-wide, causing hundreds of thousands of casualties annually. Since drug-resistant malaria parasites are spreading and mosquitoes that transmit the parasites are becoming increasingly resistant to insecticides, other tools such as efficacious vaccines are urgently needed. Most malaria vaccines that are currently being developed target a single life-cycle stage and aim to prevent either infection and disease or transmission to mosquitoes. Targeting multiple stages with a single vaccine is an attractive concept as it could prevent disease and simultaneously contribute to malaria eradication. We therefore developed multistage immunogens and tested these in mouse immunogenicity studies. Fusion proteins that contain (fragments of) *Plasmodium falciparum* circumsporozoite protein (PfCSP) and transmission blocking vaccine candidates Pfs48/45 and Pfs230 were designed and expressed as soluble proteins in *Lactococcus lactis*. The proteins were purified with a simple two-step process and used to immunize groups of outbred mice. Reference groups were immunized with single antigen constructs. All constructs elicited high antibody titers and no antigenic competition between the different components of immunogens was observed. Importantly, sera raised against immunogens that contain PfCSP reduced sporozoite invasion of a human hepatoma cell line >80% at 1:20 dilution. Furthermore, sera raised against immunogens that contain Pfs48/45 and Pfs230 reduced transmission of parasites to mosquitoes >90% at 1:9 dilution in standard membrane-feeding assay. Altogether we produced a panel of promising vaccine candidates and based on expression yield, immunogenicity and functional responses we selected a new candidate for further preclinical characterization and development.

1248

RECOMBINANT FULL-LENGTH CIRCUMSPOROZOITE PROTEIN-BASED VACCINE FOR *PLASMODIUM FALCIPARUM* IS HIGHLY IMMUNOGENIC AT LOW DOSES IN PHASE 1 TESTING AMONG HEALTHY BALTIMORE PARTICIPANTS

DeAnna J. Friedman-Klabanoff[1], Andrea A. Berry[1], Mark A. Travassos[1], Catherine Cox[2], Yingjun Zhou[2], Karen L. Kotloff[1], Annie X. Mo[3], Effie Y. Nomicos[3], Gregory A. Deye[3], Marcela F. Pasetti[1], Matthew B. Laurens[1]

[1]*Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States,* [2]*Emmes, Rockville, MD, United States,* [3]*Parasitology and International Programs Branch, Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States*

Plasmodium falciparum circumsporozoite protein (CSP) is a major sporozoite surface protein and a key target of pre-erythrocytic malaria subunit vaccines. RTS,S, the most advanced malaria vaccine to date, includes a truncated version of CSP. A full-length recombinant CSP (rCSP) based strategy may improve the modest efficacy provided by RTS,S, which lacks a region critical to sporozoite attachment and hepatocyte invasion. We initiated a first-in-human Phase 1 clinical trial of a full-length rCSP-based vaccine with and without adjuvant, Glucopyranosyl Lipid A-liposome *Quillaja saponaria* 21 formulation (GLA-LSQ) and present interim results. We enrolled 30 healthy, malaria-naïve Baltimore participants aged 18-45 years old to determine the safety and immunogenicity of rCSP administered with and without GLA-LSQ on a 1-, 29-, and 85-day schedule. Three groups of 10 participants each received 10 µg rCSP + GLA-LSQ (Group 1), 30 µg rCSP + GLA-LSQ (Group 2), or 30 µg rCSP alone (Group 3), respectively. Primary endpoints included solicited systemic and local events, related severe laboratory adverse events, related severe unsolicited adverse events, serious adverse events and adverse events of special interest. Secondary endpoints included anti-CSP IgG geometric mean and fold-rise from baseline. Six participants (20%) experienced related solicited systemic events, all mild/moderate, which did not cluster by group. Thirteen participants (43.3%) experienced solicited local events, all mild. No related laboratory adverse events occurred. Compared to baseline, geometric mean anti-rCSP IgG titer increased more in Groups 1 and 2 (54.7-fold and 49.4-fold, respectively) than in Group 3 (14.3-fold), which suggests that GLA-LSQ boosted responses. Notably, Groups 1 and 2 had similar fold increases in anti-rCSP IgG, despite the higher rCSP dose in Group 2. We conclude that rCSP/GLA-LSQ demonstrated a favorable safety, tolerability, and immunogenicity profile. The low adverse event rate and promising immunogenicity support next steps in clinical product development and confirm the immunostimulatory capacity of this relatively new adjuvant.

1249

IDENTIFYING POTENTIAL LIVER-STAGE VACCINE CANDIDATES AGAINST THE MALARIA PARASITES *PLASMODIUM FALCIPARUM* AND *P. VIVAX*

Justin L. Nicholas, Sai Iata De, Francis B. Ntumngia, Courtney Herman, Andreas Seyfang, John H. Adams

University of South Florida, Tampa, FL, United States

Plasmodium sporozoites invade hepatocytes prior to the blood-stage infection that causes disease. Therefore, targeting the pre-erythrocytic (PE) stage represents an ideal therapeutic target as it is a critical life-cycle bottleneck and precedes clinical symptoms. There still exists a gap in the availability of antigens targeting PE stage, especially for vaccines to prevent vivax malaria. Antigens that induce strain-transcending, broadly neutralizing antibodies can be utilized to enhance the efficacy of a potential PE vaccine. We hypothesize that functional neutralizing antibodies to key PE targets will interrupt essential sporozoite invasion mechanisms preventing or arresting development. To guide the selection of potential vaccine candidates, we identified infection-related targets that are upregulated with infectivity and accessible to neutralizing antibodies to block infection. Amongst these are Surface sporozoite protein 3 (SSP3), sporozoite surface protein essential for liver-stage development (SPELD) and thrombospondin-related anonymous protein (TRAP). Antisera developed to recombinant proteins of these antigens are being used to characterize localization and distribution in sporozoite and assess their potential vaccine efficacy in *in vitro* neutralizing assays. These results are the first steps in identifying potential PE vaccine candidates.

1250

MALARIA TRANSMISSION MEASURED BY DIRECT SKIN FEEDING IN SCHOOL-AGE CHILDREN AS AN ACTIVITY ENDPOINT FOR A TRANSMISSION BLOCKING VACCINE IN DONEGUEBOUGOU, MALI

Daman Sylla[1], Adama Sacko[1], Jen C. Hume[2], Abdrahamane Fofana[1], Heather Goodman[2], Boubacar Coulibaly[1], Moussa Diallo[1], Lakamy Sylla[1], Daouda Ouologuem[1], Sale Sidibe[1], Gaoussou Fofana[1], Toumany Konate[1], Mohamed L. Diarra[1], Makan Camara[1], Issaka Sagara[1], Sara A. Healy[2], Sekou F. Traore[1], Patrick E. Duffy[2], Mamadou B. Coulibaly[1]

[1]*Malaria Research and Training Center, Bamako, Mali,* [2]*LMIV/NIAID/NIH, Bethesda, MD, United States*

A vaccine to interrupt malaria transmission (VIMT) would be a valuable tool for local elimination or eradication of this disease. Transmission blocking vaccines (TBV) induce antibodies that prevent parasite development in the mosquito after a bloodmeal and can be a VIMT component. Direct skin feeds assays involve feeding colony-raised mosquitoes directly on vaccine recipients in malaria-endemic areas as a more natural measure of TBV efficacy. In pilot community studies at our sites, we observed that older school age children most frequently transmit *P. falciparum* during DSF throughout the year. Here we will present data from a community transmission blocking vaccine trial conducted in Doneguebougou, Mali, where DSFs were conducted on individuals aged 9-18 years old starting fourteen days post third vaccination and continued every fourteen days for eight total DSFs assays. In total 380 individuals underwent DSFs with a total of 2914 feeding assays conducted whereby ~60 mosquitoes were allowed to feed directly on subject forearms. 70 infected feeds were observed (2.4 %) where at least one oocyst was observed in at least one mosquito seven days post DSFs. We will discuss the transmission dynamics by DSF metrics and results (mosquito feeding rates, survival rates, infection rates and oocyst load). These parameters will permit us to acquire information about transmission in this age group in the context of TBV activity measurements.

1251

SAFETY AND EFFICACY OF RADIATION ATTENUATED *PLASMODIUM FALCIPARUM* SPOROZOITES (PFSPZ VACCINE) IN HEALTHY AFRICAN ADULT WOMEN OF CHILDBEARING POTENTIAL IN OUELESSEBOUGOU, MALI

Halimatou Diawara[1], Sara Healy[2], Djibrilla Issiaka[1], Aye Sousa Diallo[3], Seydou Traore[1], Ibrahim H Soumbounou[1], Mamoudou Samassekou[1], Oumar Dicko[1], Oumar Attaher[1], Almahamoudou Mahamar[1], Amagana Dolo[1], Balla Diarra[1], Amatigue Zeguime[1], Souleymae Traore[1], Zonghui Hu[4], LW Preston Chuch[5], Tooba Murshedkar[5], B Kim Lee Sim[5], Peter F. Billingsley[5], Thomas L. Richie[5], Stephen L. Hoffman[5], Alassane Dicko[1], Patrick Duffy[6]

[1]*Malaria Research & Training Center, Faculty of Medicine, Pharmacy and Dentistry, University of Bamako, Mali, Bamako, Mali,* [2]*Laboratory of Malaria Immunology and Vaccinology, NIAID/NIH, Washington, WA, United States,* [3]*Laboratory of Malaria Immunology and Vaccinology, NIAID/NIH, Washington DC, WA, United States,* [4]*Biostatistical Research Branch, National Institute of Allergy and Infectious Diseases, Rockville, MD, United States,* [5]*Sanaria Inc, Rockville, MD, United States,* [6]*Laboratory of Malaria Immunology and Vaccinology, NIAID/NIH, Washington, MD, United States*

Pregnant women are highly susceptible to *Plasmodium falciparum* (Pf) malaria, leading to substantial maternal, perinatal, and infant mortality. Sanaria® PfSPZ vaccine, composed of purified, cryopreserved, radiation-attenuated sporozoites (SPZ), is an advanced malaria vaccine candidate being developed for use in pregnant women, owing in part to it is highly favorable safety profile. Studies have demonstrated PfSPZ Vaccine efficacy in endemic areas following 5 doses of $2.7x10^5$ and 3 doses of $9.0x10^5$, $1.8x10^6$, and $2.7x10^6$ PfSPZ and have explored accelerated regimens, such as 1, 8, 29 days. Accelerated regimens offer an advantage to pregnant women as they could induce protection earlier in pregnancy. This study assessed the safety and efficacy of two dosage regimens of PfSPZ Vaccine, $9.0x10^5$ and $1.8x10^6$ PfSPZ administered on a 1, 8 and 29 day schedule to women of childbearing potential using a double blind placebo-controlled design. An important objective was to immunize prior to pregnancy and then study safety during pregnancy, as the women discontinued birth control following immunization. 407 volunteers were screened in June/July, 2019 and 324 enrolled to receive artemether lumefantrine (AL) starting July 3. 300 were equally randomized to receive 9 $x10^5$ or $1.8x10^6$ PfSPZ of PfSPZ Vaccine or normal saline placebo via direct venous inoculation (DVI) at 1, 8 and 29 days. All received AL 2 weeks prior to 1st and 3rd vaccination. Vaccinations have been well tolerated with 95.9% of AEs grade 1 (n= 1366), 3.4% grade 2 (n=50), 0.7% grade 3 (n= 11) and 0% grade 4. Three participants had a grade 1 generalized pruritus considered to be possibly a hypersensitivity reaction resulting in withdrawal from future vaccinations. As of February 14, 2020, Pf infection by blood smear has occurred in 168 (58.3%) of the participants, and clinical malaria (defined as signs/symptoms of malaria and positive blood smear) in 133 (46.2%). 77 women have become pregnant after receiving at least 1 dose of PfSPZ Vaccine and will be followed to term and their infants to 1 year of age to collect safety data. The study remains blinded, and efficacy results will be presented.

1252

EXCEPTIONAL IMMUNOGENICITY OF THE R21/MATRIX-M MALARIA VACCINE CANDIDATE IN ADULTS, CHILDREN AND INFANTS IN KILIFI, KENYA

Katie J. Ewer[1], Samuel Sang[2], Duncan Bellamy[1], Amy Flaxman[1], Irene Njau[2], Kelvias Keter[2], Domtila Kimani[2], Frances Ndungu[2], Melissa Kapulu[2], Adrian V. Hill[1], Philip Bejon[2], Mehreen Datoo[1], Mainga Hamaluba[2]

[1]*Jenner Institute, Oxford University, Oxford, United Kingdom,* [2]*KEMRI-Wellcome Programme, Kilifi, Kenya*

Very high titres of induced antibody are required for subunit vaccines to generate protective immunity against malaria sporozoites. R21 in matrix-M adjuvant (R21/MM), a promising new malaria vaccine candidate, induced high titres of protective antibodies in malaria-naïve adults protected in controlled human malaria infection (CHMI) studies in the UK. We have now conducted a Phase Ib open-label, study to evaluate the safety and immunogenicity of different doses of R21/MM in adults, young children and infants in Kilifi, Kenya. 20 adults, 20 children aged 1-5, and 51 infants aged 5-12 months received 3 monthly doses of 5µg or 10µg R21, with either 25µg or 50µg of MM. Tandem repeats of NANP form the central repeat of the *P. falciparum* CS protein and antibody titres to this B cell epitope are associated with efficacy following RTS,S/AS01 vaccination. RTS,S/AS01 is more immunogenic in children in malaria endemic regions, intermediate in adults in non-malaria-endemic regions, and least immunogenic in adults in malaria endemic regions. In previous trials in UK adults, R21/MM produces antibody titres similar to those previously seen with the RTS,S/AS01 malaria vaccine. In our study, R21/MM was highly immunogenic in adults in Kilifi, with antibody levels that were 3-fold higher than UK volunteers. This contrasts with RTS,S/AS01, where antibodies were substantially lower in African adults compared with US and UK adults. Published RTS,S/AS01 titres in African infants and children are increased compared to US or UK adults by about four fold. Antibody titres to NANP induced by R21/MM reached exceptional magnitudes in the Kenyan infants, with mean titres of about 15,000 (with 5µg R21/50µg MM) and 19,000 ELISA units (with 10µg R21/50µg MM) in infants, compared to 1,200 ELISA units in UK adults showing high efficacy rates in CHMI trials. The exceptionally high immunogenicity of R21/MM-induced antibodies to NANP in African infants suggest that high level efficacy and better durability of efficacy could be observed in this key target population. Phase IIB efficacy trials are underway to assess this possibility.

1253

KNOWLEDGE, ATTITUDES, AND PRACTICES RELATED TO MALARIA TRANSMISSION-BLOCKING VACCINE ACCEPTABILITY IN BO, SIERRA LEONE: A MIXED-METHODS STUDY

Kaci D. McCoy[1], Caroline Weldon[1], Rashid Ansumana[2], Joseph M. Lamin[2], David E. Stenger[3], Kevin Bardosh[4], Kathryn H. Jacobsen[5]

[1]*CDC Southeastern Center of Excellence in Vector Borne Diseases, Gainesville, FL, United States,* [2]*Mercy Hospital Research Laboratory, Bo, Sierra Leone,* [3]*Center for Bio/Molecular Science and Engineering, Naval Research Laboratory, Washington, DC, United States,* [4]*Department of Environmental and Occupational Health Sciences, School of Public Health, University of Washington, Seattle, WA, United States,* [5]*Department of Global and Community Health, College of Health and Human Services, George Mason University, Fairfax, VA, United States*

Malaria elimination remains a challenge, with approximately 450,000 deaths occurring each year, primarily in children <5 years of age in sub-Saharan Africa (SSA). A malaria transmission-blocking vaccine (mTBV) targeting Anopheles alanyl aminopeptidase N-1 (AnAPN1) could help break the cycle of malaria transmission at the community level if coupled with existing interventions. To date, no formal assessments of mTBV acceptability in SSA have been conducted. We hypothesized that mTBVs, and the AnAPN1 mTBV in particular, would be acceptable in relatively malaria intervention-naïve communities and that increased knowledge of (i) malaria transmission, (ii) individual roles associated with transmission, and (iii) how mTBVs work by acting at the community level would lead to greater mTBV acceptability. To test this hypothesis, we conducted a mixed-methods study on the knowledge, attitudes, and practices (KAP) related to the acceptability of a mTBV in July 2019 in Bo, Sierra Leone. Participants for the quantitative survey (n = 615) were recruited based on random spatial sampling, then six focus groups and 20 individual interviews were conducted with community members purposively sampled to represent parents of young children and key community health figures, respectively. Survey respondents indicated willingness to receive an mTBV for adults (95%) and children (99%). Enablers of mTBV acceptability included history of prior vaccinations for other diseases and engagement in other malaria prevention behaviors. Perceived barriers to mTBV acceptability included vaccine cost, limited understanding of malaria parasite transmission in the community, and perception of vaccine use in children only. After being provided with education about the AnAPN1 mTBV, the majority of participants in the qualitative arm of the study agreed that the vaccine could be an acceptable component of malaria elimination interventions in their community.

1254

EXPLORING MRNA IN VITRO EXPRESSION AND IMMUNE POTENCY IN MICE USING THE MALARIA ANTIGEN, PFCELTOS

Katherine Mallory[1], Neeraja Punde[1], Ishita Waghela[1], Justin Taylor[1], Michael Sibilo[1], Cosette Schneider[1], Xiaoyan Zou[2], Leah Perazzo[1], Sri Hadiwidjojo[2], Tatyana Savransky[1], Paulo Lin[3], Chris Janse[4], Ying Tam[3], **Evelina Angov**[1]

[1]*Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [2]*Naval Medical Research Center, Silver Spring, MD, United States,* [3]*Acuitas Therapeutics, Vancouver, BC, Canada,* [4]*Leiden University Medical Center, Leiden, Netherlands*

The secreted malarial protein, Cell-Traversal protein for Ookinetes and Sporozoites (CelTOS), is highly conserved among *Plasmodium* species, and plays a role in the host cell traversal and invasion. Originally identified using proteomic approaches, CelTOS induced significant IFN-γ production in PBMCs from radiation attenuated sporozoite-immunized, malaria-naïve human subjects. In the rodent model, recombinant full-length CelTOS/adjuvant combinations induced sterile protection, and in some studies, functional antibodies having invasion inhibition and transmission blocking activities. While these initial findings may be compelling, CelTOS has not demonstrated the potential as an effective vaccine candidate, using conventional vaccine approaches. Here, we report on the mRNA vaccine technology as a means to shape humoral and cell-mediated immune responses using this antigen. Several molecular constructs were developed to assess the impact of these coding modifications on expression levels. Factors tested include signal sequence, codon usage, N-glycosylation, and nucleoside modifications. Using *in vitro* transfection experiments as a pre-screen, we assessed the quality of the expressed target relative to homogeneity, cellular localization, and durability of expression levels. The optimal mRNA sequences were encapsulated in lipid nanoparticles (LNPs) and used as an immunogen in mice to assess both humoral and cellular immune responses. Our preliminary findings suggest that mRNA/LNP while potent for induction of high levels of antigen-specific cellular immune responses in mice, was less capable of inducing high levels of antibodies compared to other conventional vaccine approaches.

1256

INHIBITORY ACTIVITY OF THE JUNCTIONAL AND MAJOR REPEAT ANTIBODIES AGAINST *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PROTEIN

Merricka Livingstone, Farhat Khan, Adrian H. Batchelor, **Sheetij Dutta**

WRAIR, Silver Spring, MD, United States

Large populations around the world continue to live under the threat of contracting malaria caused by a mosquito borne parasite *Plasmodium falciparum*. Abundantly present on the surface of the malaria sporozites, the circumsporozoite protein (CSP) contains four amino acid repeats comprising a short junctional sequence (NPDP-NANP-NVDP) followed by 25-42 copies of poly-NPNA. NPNA repeat region of CSP is highly flexible and contains a myriad of secondary structures including type I β-turns. Monoclonal antibodies (mAb) that target the NPNA repeats have been isolated from recipients of a recombinant CSP vaccine - RTS,S (e.g. mAb 317 and mAb 311). Individuals with naturally exposed to *P. falciparum* also elicit anti-NPNA mAbs (e.g. mAb 580 and mAb 663). An irradiated whole sporozoite vaccine PfSPZ (Sanaria) protects by inducing CSP antibodies, however the most potent PfSPZ antibodies show dual-specific bind to the junctional epitope NPDPNANPNVDPNAN and the poly-NPNA repeats (e.g mAb CIS43 and MGG4). We conducted a systematic study comparing the biological activities of mAbs that preferably bind to the junctional region and mAbs that bind to the poly-NPNA region. We also used a tobacco mosaic virus (TMV)-particle platform, to display the junctional and poly-NPNA repeat epitopes of CSP to determine the comparative protective activity of the respective polyclonal antibodies. Overall, our data has direct implications for the design of second generation CSP vaccines and novel immune-prophylactics.

1257

CLINICAL AND COST-EFFECTIVENESS OF A LONG-LASTING FORMULATION OF MICROBIAL LARVICIDES ON MALARIA TRANSMISSION IN WESTERN KENYA: A CLUSTERED, BLOCK-RANDOMIZED, CONTROLLED, CROSSOVER TRIAL

Guofa Zhou[1], Caroline Watts[2], Xiaoming Wang[1], Ming-chieh Lee[1], Daibin Zhong[1], Harrysone Atieli[3], Andrew K. Githeko[4], Virginia Wiseman[5], Guiyun Yan[1]

[1]*University of California at Irvine, Irvine, CA, United States,* [2]*Kirby Institute for Infection and Immunity in Society, Sydney, Australia,* [3]*Maseno University, Kisumu, Kenya,* [4]*KEMRI, Kisumu, Kenya,* [5]*University of New South Wales, Sydney, Australia*

A slow-release, long-lasting microbial larvicides was tested to improve malaria control against insecticide resistance and outdoor transmission. We conducted a two-arm clustered block-randomized controlled trial using a crossover design of 34 clusters in Kakamega and Vihiga counties, western Kenya. We treated intervention clusters with one round of slow-release microbial larvicides, *Bacillus thuringiensis israelensis* (*Bti*) and *Bacillus sphaericus* (*Bs*), a boost after 3 months, then a crossover after 8 months of no treatment. The primary outcome was the number of clinically

confirmed malaria cases and health-care costs. We monitored indoor vector abundance using CDC light traps and larval abundance using a cohort of 300 randomly selected habitats. A total of 198,119 individuals were selected for clinical malaria surveillance. The malaria incidence rate after 1 month was lower in intervention group compare to control group (adj. incidence ratio 0.82, 95% CI 0.75-0.90; p<0.0001). Efficacy peaked at 3 months after *Bti/Bs* application (0.51, 0.46-0.57; p<0.0001) and lasted 5 months (0.85, 0.78-0.93; p=0.0004. Based on 3034 cases of malaria averted over a 5-month period, health system savings are estimated at USD 61,361 (95%CI 44,447-63,792). Larviciding effectively reduced indoor vector abundance for 4-6 months (adj. vector density ratio 0.41, 95% CI 0.28-0.60; p<0.0001) and habitat larval and pupal production for at least 4-5 months, with the highest reduction occurring in 3rd-4th instar larvae at 1-3 months of larviciding (0.19, 0.17-0.20; p<0.0001). Long-lasting microbial larvicide can could be used as a complementary measure alongside long-lasting insecticidal nets in areas with high vector insecticide resistance, and outdoor transmission.

1258

METABOLIC PLASTICITY OF *PLASMODIUM FALCIPARUM* IN THE *ANOPHELES* FEMALE SHAPES PARASITE TRANSMISSION

W. Robert Shaw[1], Inga E. Holmdahl[1], Maurice A. Itoe[1], Kristine Werling[1], Meghan Marquette[1], Douglas G. Paton[1], Naresh Singh[1], Amy A. Deik[2], Allison R. Demas[3], Liliana Mancio-Silva[3], Sandra March[3], Clary B. Clish[2], Sangeeta N. Bhatia[3], Caroline O. Buckee[1], Lauren M. Childs[4], Flaminia Catteruccia[1]

[1]*Harvard T.H. Chan School of Public Health, Boston, MA, United States,* [2]*Broad Institute of MIT and Harvard, Cambridge, MA, United States,* [3]*Massachusetts Institute of Technology, Cambridge, MA, United States,* [4]*Virginia Polytechnic Institute and State University, Blacksburg, VA, United States*

The extrinsic incubation period (EIP) of *Plasmodium* parasites, the time taken for their sporogonic development within the *Anopheles* mosquito, is a key determinant of malaria transmission; faster developing parasites are more likely to be transmitted to the next human host given the relatively short lifespan of mosquitoes. However, despite its relevance, relatively little is known regarding factors that influence the EIP, or how these regulators could be manipulated to block parasite transmission. Here we show that in *Anopheles gambiae*, the major African malaria vector, *P. falciparum* parasites modulate their growth and adjust their EIP in response to a female's reproductive investment and blood feeding behavior. We find that when investment in egg development is low or experimentally reduced via a number of different mechanisms, parasites exploit circulating mosquito lipids to develop more quickly, resulting in a faster transmission cycle. Faster parasites that reach the salivary glands sooner nevertheless maintain full infectivity to human hepatocytes, as determined by *in vitro* quantification of exoerythrocytic forms. Parasites also shorten their EIP when mosquitoes feed a second time during sporogony, as is typical in natural field settings, suggesting transmission efficiency, as measured by the basic reproduction number R_0, is systematically underestimated in malaria models. Combined, these data reveal that *P. falciparum* has a plastic interaction with mosquito metabolism, with profound implications for current models of malaria transmission and vector control strategies aiming to lower mosquito fecundity.

1259

MAKE DIVERSITY MEASURES GREAT

Arlinda Sadiku[1], Vincent Appiah[2], Kristan Alexander Schneider[1]

[1]*Hochschule Mittweida, Mittweida, Germany,* [2]*West African Centre for Cell Biology of Infectious Pathogens, University of Ghana, Greater Accra Region, Ghana*

Diversity measures have manifold applications in infectious diseases. Patterns of genetic diversity are informative about disease transmissions within or across endemic regions, or evolutionary processes, e.g., the emergence and spread of drug resistance. Similarly, diversity of disease vector species is crucial for vector-control interventions and climate-related range shifts of endemic areas, particularly in the context of global warming. Despite their range of applications, in practice choosing an appropriate measure is difficult, as a plethora of diversity measures exists throughout disciplines, which are difficult to compare across studies. This is also true because many measures are simple transformations of each other, leading to numerical values at different scales. While many measures have been criticized for their drawbacks, one fact has been greatly overlooked: almost all common diversity measures (or simple transformations) fall into the same general class, subsumed by a one-parameter family of measures. By reporting a continuum of diversity measures, as a function of a tilting parameter, a more complete picture is obtained, which subsumes all advantages of competing diversity measures. Based on a solid theoretical foundation, we adapt the ecological concept of alpha-, gamma- and beta- diversity, to obtain a framework that allows studying patterns of diversity (genetic, ecological) among sub-populations at different temporal and spatial scales. The term subpopulations can be interpreted at different levels of granularity maximizing the potential applications of the methods. Our approach allows studying diversity patterns by a simple graphical approach. As an example, we apply the methods to study genetic diversity of *Plasmodium falciparum* in the context of (i) evolution of drug resistance in one endemic location, particularly the patterns of SP-resistant haplotypes in a temporal context in a population in Kenya, (ii) dispersal of drug-resistance in several populations across Africa, and (iii) transmission patterns across several areas in Columbia.

1260

ENTOMOLOGICAL SURVEILLANCE OF MALARIA VECTOR POPULATIONS AND RESISTANCE AT SEVEN SITES IN KINSHASA PROVINCE, DRC: A LONGITUDINAL STUDY

Thierry Lengu Bobanga[1], Melchior Kashamuka Mwandagalirwa[2], Fabien Vulu[1], Pitshou Mampuya Luzolo[1], Mitterrand Moyo[1], Joseph Atibu[2], Kristin Banek[3], Jonathan B. Parr[3], Jonathan J. Juliano[3], Steve R. Meshnick[3], Antoinette Kitoto Tshefu[2]

[1]*Département de Médecine Tropicale, Faculté de Médecine, University of Kinshasa, Kinshasa, Democratic Republic of the Congo,* [2]*Ecole de Santé Publique, Faculté de Médecine, University of Kinshasa, Kinshasa, Democratic Republic of the Congo,* [3]*University of North Carolina-Chapel Hill, Chapel Hill, NC, United States*

The Democratic Republic of the Congo (DRC) has one of the highest burdens of malaria in Africa, with 97% of the population living in high-risk zones. To ensure effective malaria control, information is needed on local vector species and their susceptibility to insecticides. The data presented are part of an ongoing longitudinal study designed to measure malaria transmission and prevalence at seven sites in Kinshasa province. Surveys were conducted every three months from April 2018 to January 2020 using US Centers for Disease Control and Prevention (CDC) light traps from 6 PM to 6 AM during three consecutive days and by Pyrethrum Spray Catch (PSC) on the fourth day from 6 to 7 AM. *Anopheles* species and density were recorded by site. Larvae were captured every six months, reared in the lab, and tested for insecticide susceptibility using the WHO bioassay. A total of 981 *Anopheles* were captured over the study period. The most common species collected was *Anopheles gambiae* (76.7%) in all sites, but both *A. paludis* (21.5%) and *A. funestus* (1.8%) were also found. *A. paludis* were only caught during the PSC. The Anopheles density varied by village, catch time, year, and season (rainy vs. dry). The overall density during 2019 was higher than in 2018 (61.8% vs. 38.2%). WHO susceptibility test results showed evidence of *A. gambiae* insecticide resistance (<90% susceptibility) to both DDT and pyrethroids (permethrin, deltamethrin, and alphacypermethrin). The findings presented suggest an increase in malaria vectors over time, as well as a lack of susceptibility to WHO-recommend insecticides for malaria control. For malaria control to be effective, entomological surveillance data is essential for the selection of appropriate vector control strategies, monitoring of control progress, and improving our understanding of malaria transmission dynamics.

1261

MALARIA ENTOMOLOGY INDICES SUGGEST THE NEED TO CURB OUTDOOR TRANSMISSION AT THREE ECOLOGICAL ZONES IN NIGERIA

Adedapo O. Adeogun[1], Mohammed B. Audu[2], Phillip O. Oyale[2], Israel K. Olayemi[3], Monsuru A. Adeleke[4], Abdulsalami M. Yayo[5], Abduljalal Ado[5], Tolulope A. Oyeniyi[1], Abiodun K. Olakiigbe[1], Mamudu Omo-Eboh[2], Chioma N. Amajoh[1], Samson T. Awolola[1]

[1]*Center for Vector Control, Nigerian Institute for Medical Research, Yaba, Nigeria,* [2]*National Malaria Elimination Program, Federal Ministry of Health, Abuja, Nigeria,* [3]*Department of Animal Biology, Federal University of Technology, Minna, Nigeria,* [4]*Department of Zoology, Osun State University, Osogbo, Nigeria,* [5]*Center for Infectious Disease Research, Bayero University Teaching Hospital, Kano, Nigeria*

The use of Long-Lasting Insecticide Nets (LLINs) is being scaled up in Nigeria as the major vector control intervention in the country. Entomological indices are part of surveillance system, and major determinants to vector control interventions. Here we provide information on entomological indices as risk factors for indoor and outdoor malaria transmission in three ecological zones in Nigeria. Anopheline mosquitoes were collected indoors and outdoors monthly from October, 2018 to September, 2019 using modified Baited CDC light traps in three zones: Kano (Sudan Savannah), Niger (Guinea Savannah) and Osun (Forest). Anophelines were identified morphologically and through Polymerase Chain Reaction (PCR). Sporozoite infectivity was determined using Enzyme Linked Immunosorbent Assay (ELISA). Human Biting Rate (HBR), Sporozoite Infection Rate (SIR) and Entomological Inoculation Rates (EIR) were calculated as entomological risk factors indoors and outdoors. Statistical analysis were conducted using t-test to compare variables at a significant level of $P < 0.05$. A total of 1955, 1717 and 159 Anopheline mosquitoes were collected in Kano, Niger and Osun States respectively. Ten anopheline species (*Anopheles gambiae*, *An. coluzzii*, *An. arabiensis, An. funestus s.l.*, *An. squamosus*, *An. coustani*, *An. rufipes*, *An. obscurus*, *An. longipalpis*, *An. pharaoensis*) were found in Kano, four species: *An. gambiae*, *An. coluzzii, An. arabiensis* and *An. coustani* in Niger and six: *An. gambiae*, *An. coluzzii, An. arabiensis, An. funestus s.l.*, *An. squamosus* and *An. coustani* in Osun. Indoor biting rate was significant only in Osun (t=3.090, p=0.010, df=11). Sporozoites infection rate was not significant for indoor and outdoor across the three States (Kano: t=0.549, p=0.594, df=11 for Kano; t=-1.475, p=0.168, df=11 for Niger; t=0.846, p=0.416, df=11 for Osun) and consistent with the EIRs at each of the site. Taken together, the risk of malaria transmission was the same indoors and outdoors in the three states which emphasizes the need for equal attention to outdoor transmission in these zones in Nigeria.

1262

ANOPHELES SPECIES INVOLVED IN MALARIA TRANSMISSION IN THE COLOMBIAN PACIFIC REGION

Stefani A. Piedrahita, Margarita M. Correa

Grupo de Microbiologia Molecular, Escuela de Microbiología, Universidad de Antioquia, Medellín, Colombia

In Colombia, malaria is an important problem of public health and the Pacific region is currently the most malarious endemic area in the country. The detection of *Plasmodium* infected mosquitoes is an important parameter for the incrimination of vectors in malaria transmission. Therefore, the aim of this study was to detect natural infection by *Plasmodium* parasites in *Anopheles* mosquitoes collected in 16 localities of the Colombian Pacific region. A total of 1,821 mosquitoes were evaluated by genus-specific nested PCR and four were detected infected. Three corresponded to the main vectors, *Anopheles albimanus* infected with *P. falciparum*, *A. darlingi* with *P. vivax, A. nuneztovari* with *Plasmodium* spp. In addition, the suspected local vector *A. calderoni* was detected with *Plasmodium* spp. These results indicate that malaria transmission in the Pacific region is mainly maintained by the Colombian main malaria vectors; however, their distribution varies depending on the locality, which suggests the importance of applying control interventions that are locality/vector-specific. Further studies on feeding behaviors and host preferences will allow identifying species with opportunistic and/or anthropophilic behavior, of relevance for the type of interventions to be implemented.

1263

PREDICTING COMMUNITY-LEVEL IMPACT OF TRANSFLUTHRIN-TREATED EAVE RIBBONS ON MALARIA TRANSMISSION FROM SEMI-FIELD STUDIES

Adrian E. Denz[1], Margaret M. Njoroge[2], Mgeni M. Tambwe[3], Clara Champagne[1], Matt Worges[4], Fredros Okumu[3], Joop J. van Loon[5], Josh Yukich[4], Thomas A. Smith[1], Alexandra Hiscox[6], Adam Saddler[1], Ulrike Fillinger[2], Sarah J. Moore[3], Nakul Chitnis[1]

[1]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [2]*International Centre of Insect Physiology and Ecology, Human Health Theme, Nairobi, Kenya,* [3]*Ifakara Health Institute, Environmental Health and Ecological Sciences Department, Ifakara, United Republic of Tanzania,* [4]*Tulane University School of Public Health and Tropical Medicine, Center for Applied Malaria Research and Evaluation, New Orleans, LA, United States,* [5]*Wageningen University, Laboratory of Entomology, Wageningen, Netherlands,* [6]*ARCTEC, London School of Hygiene & Tropical Medicine, London, United Kingdom*

Semi-field experiments with human landing catch (HLC) measurements are an important step in the development of novel vector control interventions against outdoor transmission of malaria since they provide good estimates of personal protection. However, it is often unfeasible to determine whether the reduction in HLC counts is due to mosquito mortality or repellency. Due to the vastly differential impact of repellency and mortality on transmission, this means that the community-level impact of spatial repellent products could not be estimated from such semi-field experiments. We present a new stochastic model that is able to estimate the full effect on mosquito host-seeking behaviour of a product, based only on time-stratified HLC data from controlled semi-field experiments, distinguishing between repellency on one hand and either mortality or disarming (preventing mosquitoes from host-seeking until the next night) on the other. This allows us to estimate the impact of the product on the vectorial capacity of the given *Anopheles* species, and hence the community-level effect, using an existing mathematical model. Using an individual-based simulation of malaria disease (OpenMalaria), we can predict the impact of the product on malaria incidence and mortality and investigate its interaction with insecticide treated nets (ITNs) and indoor residual spraying (IRS). With this methodology, we analysed data from recent semi-field studies in Kenya and Tanzania on the impact of transfluthrin-treated eave ribbons, the odour-baited Suna trap and their combination (push-pull system) on outdoor HLC of *Anopheles arabiensis* in the peridomestic area. Complementing previous analyses of personal protection, we found that the transfluthrin-treated eave ribbons act mainly by killing or disarming mosquitoes, and hence provide both user- and community-level protection. The odour-baited Suna trap had minor impact. The results of our analysis suggest that a substantial reduction in vectorial capacity and malaria case incidence might be achieved even with low coverage of transfluthrin-treated eave ribbons.

1264

PRELIMINARY ESTIMATES OF THE COST-EFFECTIVENESS OF NEXT-GENERATION INSECTICIDE-TREATED BEDNETS IN VARIED RESISTANCE AND TRANSMISSION SETTINGS

Joshua Yukich[1], Ellie Sherrard-Smith[2], Thomas S. Churcher[2], Peder Digre[3], Molly Robertson[4]

[1]*Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States,* [2]*Imperial College London, London, United Kingdom,* [3]*PATH, Seattle, WA, United States,* [4]*PATH, Washington, DC, United States*

The effectiveness of malaria vector control tools is threatened by increasing insecticide resistance in vector populations. Insecticide-treated bednets

(ITNs) are now available that either have more than one active ingredient in addition to pyrethroids or contain a pyrethroid and the insecticide synergist piperonyl butoxide. These next-generation ITNs have been shown to be effective against pyrethroid-resistant mosquitoes and are alternatives to standard, pyrethroid-only long-lasting insecticidal nets (LLINs). We produced preliminary estimates of effectiveness, cost, and cost-effectiveness for the mass distribution of these ITNs compared to standard LLINs for varied transmission and insecticide resistance settings. A transmission model for *Plasmodium falciparum* was parameterized using experimental hut data quantifying the entomological impact of next-generation ITNs. A relationship was fit between the proportion of mosquitoes surviving the World Health Organization bioassay test and the standard LLIN induced mosquito mortality from experimental hut data. Then associations between standard LLIN mosquito mortality and the next-generation ITN mosquito mortality from trials determined their additional benefit. These relationships are combined with the feeding inhibition and deterrence measured in experimental huts to determine parameters used in the model. The efficacy can then shift as the level of pyrethroid resistance changes. The transmission model is used to project the clinical cases averted by each net type relative to standard LLINs, assuming the same net coverage. Total cost of deployment, including uncertainty intervals, were estimated using data from a systematic review and incremental cost-effectiveness ratios were calculated. Results will be updated and validated using randomized controlled trials and observational studies which are currently underway. Preliminary estimates indicate that the optimal choice of ITN is highly dependent on the baseline transmission and resistance context indicating that local decisions will be needed to make the most of scarce resources in future bednet campaigns.

1265

EFFICACY OF ENTOMOLOGICAL INOCULATION RATES IN NCHELENGE, A MALARIA HOLOENDEMIC DISTRICT IN NORTHERN ZAMBIA

Mbanga Muleba[1], James James[1], David Mbewe[1], Francis K. Mulenga[1], William J. Moss[2], Jennifer C. Stevenson[3], Douglas E. Norris[4]

[1]*Tropical Diseases Research Centre, Ndola, Zambia,* [2]*Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [3]*Macha Research Trust, Choma, Zambia,* [4]*The W. Harry Feinstone Department of Molecular Microbiology and Immunology, Johns Hopkins Malaria Research Institute, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States*

The entomological inoculation rate (EIR) is an important indicator of intensity of malaria transmission in an area. Reduction in EIR is one of the indices used to measure impact following vector control activity. The EIR is a product of the human biting rate and parasite sporozoite rate in malaria vectors. Methods for measuring EIR lack standardization across a spectrum of epidemiological settings. The most direct method of measuring biting rates is the human-landing catch (HLC). The HLC is believed to catch mosquitoes actively searching for a human blood meal. Risk of malaria infection and other vector-borne diseases during HLCs becomes an ethical issue. We used CDC light-traps, one of the alternatives to HLCs, set next to people indoors to determine biting rates. We used ELISA-based method to determine sporozoite rates and estimated the EIRs on two islands and the mainland areas of Nchelenge District, northern Zambia in January, March, July, October 2015 and January 2016. We obtained 18 months of concomitant malaria incidence data from health centres servicing the three individual collection sites. Kilwa Island had the highest incidence of malaria followed by the mainland with Chisenga Island registering the lowest incidence. In contrast the estimated EIRs were highest on Chisenga Island followed by the mainland, then Kilwa Island. In an area with high vector counts, the discrepancy between estimated EIR and malaria cases may be due to the increased use of nets or other vector control measures, which could reduce case incidence. Local factors including the ecology, demographics, malaria control use and mosquito bionomics need to be considered in the estimation and interpretation of the EIR in Nchelenge, as well as an understating of how this was estimated. Measured in a consistent standardized way, EIRs are likely more useful as indicators of change over time in a given location than as a comparison measure across different settings. Monitoring how EIRs are affected will be important in the evaluation of the scale-up of the malaria control package that will include indoor residual spraying and community case management on the islands of Nchelenge.

1266

AN OPERATIONAL REAL-TIME DASHBOARD TO TRACK VECTOR CONTROL ACTIVITIES ON BIOKO ISLAND

Olivier Tresor Donfack Sontsa[1], Carlos A. Guerra[2], Emily Hilton[3], Brent Atkinson[2], Guillermo A. Garcia[2]

[1]*MCDI, Malabo, Equatorial Guinea,* [2]*MCDI, Silver Spring, MD, United States,* [3]*Institute of Health Metrics and Evaluation, University of Washington, Seattle, WA, United States*

The Bioko Island Malaria Elimination Project (BIMEP) has delivered 15 years of vector control, case management and social behavioral change communication. Decision-making and intervention targeting are guided by data obtained through a robust monitoring and evaluation system. In 2020, an integrated vector control strategy was adopted in an attempt to further reducing malaria prevalence that had stalled since 2015 and increased in 2019. The strategy included the expansion of indoor residual spraying to ~ 34,000 households, a top-up distribution of long-lasting insecticidal bed nets to ~20,000 households, larval source management in high prevalence areas and an integrative malaria sensibilization package. With such a huge deployment and limited resources, the use of data can improve allocation, optimize productivity and monitor vector control coverage. The BIMEP developed a comprehensive dashboard to facilitate real-time assessment of work progress, productivity and coverage. The dashboard used three main components: (1) a *coverage-map* dashboard, which displays spatial coverage at 100x100 m sectors (the units for vector control deployment); (2) a *productivity dashboard*, which displays productivity by field teams; and (3) an *overall metrics dashboard* that shows cumulative coverage and other general metrics over time. The dashboard sourced data directly from the BIMEP PostgreSQL server. Weekly meetings were held to visualize and interpret the data and guide the teams to make decisions accordingly. This facilitated adjusting deployment strategies in order to optimize production and meet targets. Although evidence is crucial for effective malaria vector control, timely and relevant data are often not integrated and readily available to inform decision-making. Online tools, such as the dashboards presented here, can help malaria control programs to track critical components of project performance and enhance decision-making. In order to achieve this, however, programs require to develop robust data assemblies and hosting systems in dedicated servers.

1267

HABITAT ADAPTATION OF *ANOPHELES COLUZZII* AND *AN. MELAS* TO NEGLECTED POLLUTED SWIMMING POOLS, ABANDONED BOATS AND CRAB HOLES ON BIOKO ISLAND

Godwin Fuseini[1], Michael von Fricken[2], Raul Ncogo Nguema[3], Jeremias Nzamio[1], Jordan Smith[1], Carlos A. Guerra[4], Guillermo A. Garcia[4]

[1]*MCDI, Malabo, Equatorial Guinea,* [2]*Department of Global and Community Health, George Mason University, Fairfax, VA, United States,* [3]*Ministry of Health and Social Welfare, Malabo, Equatorial Guinea,* [4]*MCDI, Silver Spring, MD, United States*

Insecticide resistance and changes in biting behavior of malaria vectors have renewed interest in larval source management (LSM) on Bioko Island as a useful supplement to the core vector control interventions of indoor residual spraying and insecticide-treated nets. Typically, anopheline breeding habitats have included small puddles, car tyre tracks, marshes and mangrove swamps, open drains and roadside ditches. *Anopheles* mosquitoes, however, can adapt to a diversity of breeding habitats due to changes in vector ecology. During an effort to characterize anopheline

breeding habitats for LSM on the island, *An. coluzzii* and *An. melas* were found breeding in a diversity of water collections. On the one hand, out of a total of 247 swimming pools identified and evaluated in the highly urbanized city of Malabo, 195 were in good conditions, 43 neglected and 9 with no access. *Anopheles coluzzii* larvae were found in 54% of the neglected swimming pools that were clogged and polluted, and were treated with a silicone-based liquid larvicide, Aquatain AMF. On the other hand, in a coastal rural area where the dominant vector species is *An. melas*, larvae were found breeding in polluted abandoned boats, crab holes and plastic spreadsheets for drying fish. Given their small size, identifying all such breeding sites for larviciding remained a challenge and could not be realized. Our findings put in evidence that changes in the urban landscape as well as in the local vector ecology has resulted in mosquitoes adapting to a diversity of breeding sites. They also highlight the necessity of continued monitoring and characterization of the breeding habitats for effective implementation of LSM.

1268

EXAMINING HUMAN BEHAVIOR TO ESTIMATE THE MALARIA PREVENTION IMPACT OF NEXT-GENERATION INSECTICIDE-TREATED BEDNETS

Federica Guglielmo[1], Chantal Ingabire[2], Adama Gansané[3], Kenzie Tynuv[4], Jenny Shannon[5], Molly Robertson[4]

[1]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [2]*University of Rwanda, Kigali, Rwanda,* [3]*Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso,* [4]*PATH, Washington, DC, United States,* [5]*PATH, Seattle, WA, United States*

Progress toward malaria control and elimination has recently stalled. The emergence and intensification of insecticide resistance among malaria vectors threatens the effectiveness of vector control tools, such as insecticide-treated bednets (ITNs) and indoor residual spraying (IRS), and has resulted in development of new tools, such as next-generation ITNs. To improve access to next-generation ITNs, the New Nets Project is supporting the scale-up of Interceptor G2® (BASF)(IG2) ITNs through a market intervention and effectiveness pilot distributions in Burkina Faso, Mozambique, Nigeria, and Rwanda. The IG2 rollout will be accompanied by data collection on factors that influence their uptake and usage, so that real-world effectiveness and cost-effectiveness of IG2 ITN deployment can be assessed. Qualitative data on human behavior will be collected to better demonstrate the public health value of next-generation ITNs. The human behavior assessment adopts a mixed-method approach, with a quantitative component nested within a broader qualitative component. The qualitative component investigates human behaviors that influence patterns of exposure, through direct observations, in-depth interviews, and focus group discussions, providing a crucial framework of analysis to the quantitative data. The quantitative component uses surveys to measure the actual time individuals spend unprotected by ITNs, including classifications of types of activities and locations. This information will aid in the modeling and cross-analysis with entomological and epidemiological data. Improved insight into human behavior surrounding ITN interventions will allow us to better understand the pathways between distribution of an intervention and disease prevention, more accurately model intervention effectiveness, and guide more effective decision-making in the promotion and distribution of ITNs.

1269

TO SUPPRESS OR TO MODIFY - EVALUATING GENE DRIVE STRATEGIES FOR MALARIA ELIMINATION THROUGH AGENT-BASED MODELING

Prashanth Selvaraj, Caitlin Bever, Edward Wenger

Institute for Disease Modeling, Bellevue, WA, United States

Gene drives result in select genes spreading through a population at a higher than normal inheritance rate leading to phenotypes associated with these genes dominating a population faster than random genetic drift. Consequently, gene drive mosquitoes have been proposed as a modality to rapidly achieve malaria elimination either by modifying mosquitoes to render them refractory to malaria transmission or by suppressing specific species of mosquitoes through sex distortion of progeny or reduced fecundity of adult mosquitoes. Both population suppression and modification methods, however, require sufficient numbers of mosquitoes to mate and propagate the desired phenotypes through the population. Seasonal variation in mosquito densities, mutation of genetic constructs as well as phenotypic fitness costs, and spatially heterogeneous establishment of gene drives may all lead to a drive failing to achieve elimination. Here, we investigate which gene drive strategies offer the most optimal path to malaria elimination in a range of transmission settings by leveraging a large scale individual-based model of malaria transmission in combination with a multi-locus, agent-based model of vector genetics that accounts for mutations and many-to-many mappings of genotypes to phenotypes. We also evaluate release conditions that will maximize the efficacy of combining suppression and modification strategies, which potentially offers a greater chance of elimination than either method deployed individually in settings with multiple bottlenecks to drive establishment.

1270

MITIGATING THE EFFECTS OF A CYCLONE IN MOPEIA, MOZAMBIQUE

Emily Beylerian[1], Joseph Wagman[2], Carlos Chaccour[3], Aina Casellas[4], Amilcar Nacima[5], Eldo Elobolobo[5], Binete Savaio[6], Abuchahama Saifodine[7], Christen Fornadel[8], Jason Richardson[8], Baltazar Candrinho[9], Molly Robertson[2]

[1]*PATH, Seattle, WA, United States,* [2]*PATH, Washington, DC, United States,* [3]*ISGlobal, Barcelona Centre for International Health Research, Hospital Clínic, Universitat de Barcelona; Centro de Investigação em Saúde de Manhiça, Barcelona, Spain,* [4]*ISGlobal, Barcelona Centre for International Health Research, Hospital Clínic, Universitat de Barcelona, Barcelona, Spain,* [5]*Centro de Investigação em Saúde de Manhiça, Maputo, Mozambique,* [6]*PATH, Maputo, Mozambique,* [7]*President's Malaria Initiative, United States Agency for International Development, Maputo, Mozambique,* [8]*IVCC, Liverpool, United Kingdom,* [9]*Programa Nacional do Controlo da Malaria, Maputo, Mozambique*

As mosquito resistance to pyrethroids increases and additional third-generation indoor residual spraying (3GIRS) products, which are effective against pyrethroid-resistant vectors and have a targeted residual efficacy of at least 6 months, are introduced to the market, it is important to evaluate the effectiveness of new, 3GIRS products as potential components of rotation strategies for insecticide resistance management. In the Mopeia District of Zambezia Province, Mozambique, a high transmission area with pyrethroid-resistant *An. funestus*, a two-armed cluster-randomized trial was conducted from 2016 to 2018 to evaluate the impact of annual IRS with a microencapsulated formulation of pirimiphos-methyl (Actellic®300CS), a 3GIRS product, in combination with standard long lasting insecticide nets (LLINs), compared to LLINs only. In late 2018, after the conclusion of the two-year trial, all villages in Mopeia, including the non-IRS arm, received IRS using clothianidin (SumiShield™50WG, Sumitomo Chemical). During the following 2019 transmission season, Cyclone Idai devastated the region of central Mozambique. In order to explore the impact of transitioning to SumiShield™50WG, a difference-in-differences approach has been used to compare the seasonal incidence changes after the non-IRS Mopeia study arm transitioned to SumiShield with incidence changes in four neighboring districts with malaria transmission patterns historically similar to Mopeia that did not receive IRS in any study year. Likely because of the impacts of the cyclone, malaria incidence increased from 2018 to 2019 across the entire region, including in the district of Mopeia. However, preliminary results indicate that in the Mopeia study arm that transitioned from no-IRS to SumiShield, the increase in incidence was significantly smaller compared to the surrounding comparator districts without any IRS (a 31% smaller increase, $p = 0.022$), suggesting that IRS with SumiShield provided some protective effect against the rising malaria incidence rates during the immediate post-cyclone period.

1271

EVIDENCE OF *PLASMODIUM VIVAX* TRANSMISSION IN A HIGHLY *P. FALCIPARUM* ENDEMIC REGION OF CENTRAL MOZAMBIQUE

Joseph Wagman[1], Molly Robertson[1], Laurence Slutsker[2], Rose Zulliger[3], Abuchahama Saifodine[4], Rodaly Muthoni[5], Kenyssony Varela[5], Dereje Dengela[6], Aklilu Seyoum[6], Christen Fornadel[7], Jason Richardson[7], Carlos Chaccour[8], Francisco Saute[9], Yvonne-Marie Linton[10], Baltazar Candrinho[11]

[1]*PATH, Washington, DC, United States,* [2]*PATH, Seattle, WA, United States,* [3]*President's Malaria Initiative, Division of Parasitic Diseases and Malaria, US Centers for Disease Control and Prevention, Maputo, Mozambique,* [4]*President's Malaria Initiative, United States Agency for International Development, Maputo, Mozambique,* [5]*Abt Associates, Maputo, Mozambique,* [6]*Abt Associates, Bethesda, MD, United States,* [7]*IVCC, Liverpool, United Kingdom,* [8]*ISGlobal, Barcelona Centre for International Health Research, Hospital Clínic, Universitat de Barcelona; Centro de Investigação em Saúde de Manhiça, Barcelona, Spain,* [9]*Centro de Investigação em Saúde de Manhiça, Maputo, Mozambique,* [10]*Walter Reed Biosystematics Unit; Smithsonian Institution, National Museum of Natural History, Washington, DC, United States,* [11]*Programa Nacional do Controlo da Malaria, Maputo, Mozambique*

Understanding the local characteristics of malaria transmission is the foundation for any successful vector control strategy. During a recent randomized trial evaluating the impact of indoor residual spraying in Mopeia District of Zambezia Province in Mozambique, DNA from multiple *Plasmodium* species was detected in multiple *Anopheles* species. A total of 26,348 anophelines were collected by light traps and human landing collections, and a subsample had the head/thorax region screened by PCR for *Plasmodium* DNA. In all, 6,216 mosquitoes from 16 different *Anopheles* species were screened. Of these, 167 (2.7%) were positive for *Plasmodium* DNA: 146 (2.3%) for *P. falciparum* (Pf), 24 (0.4%) for *P. vivax* (Pv), and 3 (< 0.1%) for co-infection with both. Of the 146 Pf-positive mosquitoes, 95.9% (n = 140) belonged to the *An. funestus* group, 1.4% (n = 2) to the *An. gambiae* complex, and 1.4% (n = 2) to the *An. coustani* group. Most (n=131) of these specimens were further identified to species using PCR. The dominant vector for both *Plasmodium* species was *An. funestus* s.s., accounting for 94.7% (n = 124) of all Pf-positive mosquitoes identified to species; 85.7% (n = 18) of all Pv-positive mosquitoes identified to species, and two of the three co-infected mosquitoes. Other vectors that tested positive for *Plasmodium* include *An. rivulorum* (n = 3 Pf; n = 1 Pv), *An. funestus* s.l. specimens that could not be identified to species (n = 13 Pf; n = 1 Pv), *An. gambiae* s.s. (n = 2 Pf; n = 2 Pv), *An. coustani* s.s. (n = 1 Pf), and *An. namibiensis* (n = 1 Pf). In Mopeia, multiple anopheline species transmit both Pf and Pv. However, *P. falciparum* is still by far the most prevalent malaria parasite in Mopeia and *An. funestus* s.s. is the dominant vector for both Pf and Pv. As control efforts reduce the burden of *P. falciparum* transmitted by *An. funestus* in Mopeia, it will be important to monitor the contribution of other malaria parasite species and vectors to the overall malaria burden.

1272

HOUSEHOLD RISK FACTORS ASSOCIATED WITH INDOOR VECTOR DENSITY IN RURAL SOUTHWEST BURKINA FASO

McKenzie Colt[1], Emmanuel Sougue[2], Lyndsey Gray[3], Catherine Neya Ebou[2], Anthony Some[2], Fabrice Some[2], Brian Foy[3], Roch Dabire[4], Sunil Parikh[1]

[1]*Yale School of Public Health, New Haven, CT, United States,* [2]*Institut de Recherche en Sciences de la Sante, Bobo-Dioulasso, Burkina Faso,* [3]*Colorado State University, Fort Collins, CO, United States,* [4]*Institut de Recherche en Sciences de la Sante, Fort Collins, CO, United States*

Malaria transmission occurs both indoors and outdoors, and housing and peri-domestic characteristics have been associated with higher risks of malaria. Previous research in Burkina Faso has demonstrated that vector density within households varies greatly, though contributing factors are less clear. In the context of a cluster-randomized trial in a rural region of southwest Burkina Faso, the associations between household structural and behavioral factors with vector density will be presented. General household surveys were completed detailing the external housing characteristics of wall and roof materials, number of individual sleeping rooms, number of individuals in the household, and LLIN use. All enrolled households in 14 villages were included [n=556 households with a median of 39.7 households per village, a median of 308 individuals per village (n~4314 total individuals), and 7.8 individuals per household]. Results showed that the primary materials for walls was earth (61.3%) or cement/plaster (34.4%) while roofs were thatch (88.3%), straw (6.1%) or earth (4.9%). There was a median of 4.9 (range 2-6) LLINs per household (0.6 nets per person per household). Following the general survey, three villages were randomly chosen from the intervention arm and 3 from the control arm for cross-sectional sampling, with four households chosen on two perpendicular transects (8 total) in each village based on them being either centrally or peripherally-located (n=48 total households). A more detailed survey was completed detailing the characteristics of each individual sleeping rooms as well as the household. There was a median 2.3 (range 1-3) sleeping rooms per household. In order to explore associations of indoor vector density with housing and inhabitant characteristics, weekly mosquito vacuum aspirations were conducted in the early mornings during the rainy season in each of the individual rooms of the 48 cross-sectional households. A more detailed analysis will be presented to highlight potential household structural and behavioral factors that determine vector density within a rural setting in southwest Burkina Faso.

1273

ROLE OF PUBLIC ENGAGEMENT IN IMPLEMENTING A LARGE-SCALE TYPHOID CONJUGATE VACCINE TRIAL IN LALITPUR, NEPAL

Ashata Dahal, Mila Shakya

Oxford University Clinical Research Unit-Nepal, Patan, Lalitpur, Nepal

Typhoid is a systemic illness caused by *Salmonella enterica* is and a public health burden in Kathmandu, which has been coined as an Enteric Fever Capital of the world. A new position paper from WHO (2018) recommends the use of typhoid conjugate vaccine (TCV) in typhoid endemic countries among children 6 months or older. However, vaccine-based control programs have not been widely implemented. As a part of Typhoid Vaccine Acceleration Consortium (TyVAC), a phase III participant-and-observer blinded, individually randomized controlled trial is being conducted in Lalitpur, Nepal with an aim to assess the protective impact of Vi-TCV. Literature shows that a sound and robust communication of the research team with communities and authorities is a key element for a successful clinical trial. Public Engagement (PE) has been an integral component of this trial to inform, communicate and collaborate with stakeholders. These engagements give an opportunity for the research team to address any study related concerns through interactions and a platform to receive feedback from the participants and stakeholders.PE has been conducted in a tiered approach. Pre study approvals, national immunization advisory committee and key government decision makers were engaged in discussion. After receiving ethical approvals, PEs have been conducted, continuously and simultaneously with the study. We conduct our 1st level engagement with the locally elected representatives and ward health implementation committee members, while, 2nd level of PE is conducted with the key community stakeholders. We conduct engagements with the guardians of study participants, community members, and at schools for 3rd level. During engagements at various levels, results of interim analysis have also been shared consecutively. Public Engagements aim to keep the stakeholders and community members informed about the trial and clarify any questions, concerns and rumors about it. Understanding and integrating socio-cultural realities along with the two-way communication at various levels has proved to be an asset in conducting and helping the community to accept the clinical trial.

1274

RETROSPECTIVE ANALYSIS OF DIARRHEAL ETIOLOGIES IN THE COUNTRY OF GEORGIA

Nino Mitaishvili[1], Tea Tevdoradze[2], Shannon Walls[1], Nino Trapaidze[1]

[1]*United States Army Medical Directorate-Georgia (USAMRD-G), Tbilisi, Georgia,* [2]*National Center for Disease Control and Public Health (NCDC), Tbilisi, Georgia*

Enteric pathogens causing acute diarrhea to pose a significant threat to military and civilian populations. United States (US) troops deployed to resource-limited countries are at elevated risk of exposure to local enteric pathogens that could degrade unit readiness. The current knowledge of enteric disease etiology in Georgia is very limited which constitutes a Force Health Protection (FHP) knowledge gap for US and allied forces assigned there. The present study aims to identify the probable cause of acute diarrhea in Georgia by retrospective molecular analysis of stool specimens from patients presenting with acute diarrheal symptoms. USAMRD-G obtained stool specimens from the Georgian NCDC that tested negative for rotavirus, norovirus and adenovirus via immunoassay to conduct the present retrospective study. Nucleic acid extracted from stool specimens were tested using a custom TaqMan Array Card platform, simultaneously detects 37 bacterial, viral, fungal, and parasitic diarrheal pathogens, developed by the Houpt Laboratory at the University of Virginia. This advanced molecular technology. USAMRD-G tested 103 samples. Pathogens were detected in 81 (78.6%) of the previously negative samples: 31 (30.1%) for single pathogens and 50 (48.5%) for co-infections. Pathogenic *Escherichia coli* were the most common bacterial pathogens detected (42.7%), followed by *Campylobacter jejuni/coli* (22.3%), *Bacteriodes fragilis* (8.7%), and *Clostridioides difficile* (3.9%). Adenovirus was the most frequent viral agent detected (15.5%), followed by rotavirus (10.7%), enterovirus (8.7%) and norovirus (5.8%). Protozoa were detected in 10.7% of samples, whereas other pathogens were identified in single cases. Twenty-two stool specimens (21.4%) were negative for all infectious agents tested. Preliminary data identify the presence of a broad spectrum of previously undetected, duty-limiting enteric pathogens that constitute a credible health threat to US and allied partners in Georgia. These and future surveillance efforts will inform military leaders when making FHP and operational planning decisions.

1275

COMPARISON OF HOST FECAL MRNAS AND STOOL INFLAMMATION BIOMARKERS IN THE EVALUATION OF ENVIRONMENTAL ENTERIC DYSFUNCTION IN AN URBAN ETHIOPIAN INFANT COHORT

Leon Espira[1], Gwenyth O. Lee[1], Brook Gesesse[2], Kaleab Baye[2], Andrew Jones[1], Nancy G. Love[1], Joseph N. Eisenberg[1]

[1]*University of Michigan, Ann Arbor, MI, United States,* [2]*Addis Ababa University, Addis Ababa, Ethiopia*

Environmental Enteric Dysfunction (EED) is a syndrome of intestinal alteration. Histologically, EED presents as a flattening of the villi, resulting in a reduction of the absorptive area of the intestine. A chronic inflammatory response is also characteristic, resulting in compromised gut wall integrity. Currently, there are no standard markers to reliably diagnose EED. The most common approach has been to use the cumbersome urinary lactulose: mannitol (L:M) test to evaluate intestinal permeability. Inflammatory biomarkers in stool samples have also been used, however, the predictive utility of these markers is unclear. To explore alternative EED biomarkers, we evaluated 4 novel fecal stool mRNAs screened for using droplet digital PCR (ddPCR): sucrase isomaltase, SI, a disaccharidase; S100A8, a regulator of the immune response; mucin 12, a component of the gastrointestinal mucous layer; and caudal homeobox 1 (CDX1), a transcription factor in intestinal differentiation. Stool samples from 136 infants aged 6-23 months from informal settlements in Addis Ababa were used for the analysis. We compared the novel mRNAs to three biomarkers that have been utilized as putative predictors of EED: alpha-1-antitrypsin (AAT), an indicator of intestinal permeability; and 2) two inflammatory biomarkers (myeloperoxidase and neopterin). In addition, we screened the stool samples for 16 enteropathogens. We examined correlations between the different biomarkers and how these related with stool pathogen loads. Biomarker-pathogen relationships were found to be enteropathogen-specific, reflecting expected physiological processes of epithelial invasion and gut dysfunction. We find that even though the biomarkers can be accurate measures of cellular damage and allow the evaluation of specific components of the inflammatory process, the relationships between the biomarkers are more nuanced. We postulate that EED is the result of multiple different cellular damage processes that may differ between individuals. Our study provides further insights into the etiology of EED and suggests considerations for the future evaluation of EED.

1276

THE PREVALENCE OF ANTIMICROBIAL RESISTANCE GENES IN ANAEROBES ISOLATED FROM HEALTHY PEOPLE IN RURAL AREAS IN VIETNAM AND JAPAN

Hanh Vu, Kaori Tanaka, Yoshimasa Yamamoto

Gifu University, Gifu, Japan

Normal non-pathogenic flora could be harming the host by acting as a reservoir of resistance determinants potentially transferable to human pathogens. To assess the distribution of antimicrobial resistance genes in anaerobes from normal flora of Vietnamese and Japanese, 135 organisms obtained from the fecal samples of 80 healthy individuals (51 Vietnamese and 30 Japanese). The identification of isolated strains was done using MALDI-TOF MS and the minimum inhibitory concentration (MIC) values were determined by using an agar dilution method based on the recommendation of the Clinical and Laboratory Standards Institute (CLSI). Nitrocefin discs were used to detect the production of β-lactamase.The presence of the resistance genes (*cepA, cfxA, cfiA, nim, ermB, ermF, ermG, linA, mefA, msrSA, tetM, tetQ, tetX, tetX1, tet36, bexA, qnrA, qnrB, qnrS* and *catA*) and one virulence gene (*bft*) was determined by standard PCR. The prevalence of resistance genes was compared with the phenotypic resistance and between countries. All the isolates tested positive for b-lactamase production. The *cepA* gene occurred at different frequencies among *Bacteroides fragilis* (100%) and non-fragilis *Bacteroides* strains (5%). All the *cfiA* (5%) was found in non-fragilis *Bacteroides* and silent. The *ermG-mefA-msrSA* combination was found in 3 strains in a total of 4 strains that harbored *msrSA*. The *bexA* gene was found in 81% of *B. thetaiotaomicron* and none of them from moxifloxacin-resistant strains. There was a statistically significant difference in the prevalence of *cepA*, *ermG, mefA* and *msrSA* between countries. There was only one *tetX1*-positive strain isolated from a Japanese. All strains tested were susceptible to metronidazole and none of them harbored *nim* gene. This study is the first report on the distribution of resistance genes in anaerobes isolated from healthy people in Vietnam and Japan. Further investigations are needed to determine resistance mechanisms of specific anaerobes and the potential transmission of resistance determinants to human pathogens.

1277

DEATH IN HOSPITALIZED SEVERELY MALNOURISHED CHILDREN PRESENTING WITH DIARRHEA & VOMITING

Abu Sadat Mohammad Sayeem Bin Shahid, Tahmina Alam, Lubaba Shahrin, Mohammod Jobayer Chisti

International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh

Vomiting in children with diarrhea especially with severe malnutrition is often associated with serious complication. However, data on outcomes of such children with vomiting are lacking. Thus, we evaluated outcomes of children with vomiting who were hospitalized with severe malnutrition & diarrhea. In this chart review, we used electronic database and evaluated children with diarrhea aged 0-59 months, admitted to the Dhaka Hospital of the International Centre for Diarrhoeal Disease Research, Bangladesh, with severe malnutrition between April 2011 and August

2012. Comparison of outcomes was made between the children with & without vomiting. The primary outcome was death. Out of 306 enrolled children with diarrhea & severe malnutrition, 51 (17%) had vomiting and 255 (83%) did not have vomiting. Baseline analysis revealed that the study children with vomiting more often had dehydration (39% vs. 12%; p<0.01), hypoglycaemia (6% vs. 1%; p=0.03) & metabolic acidosis (67% vs. 50%; p=0.03) compared to those who did not have vomiting. A total of 31 (10%) children died, 12 (24%) of them had vomiting &19 (8%) did not have vomiting. Death was significantly higher in severely malnourished diarrheal children having vomiting [12/51 (24%)] compared to those without vomiting [19/255 (8%)] (p<0.001). Using Log linear bi-nominal regression after adjusting for potential confounders such as metabolic acidosis and hypoglycaemia, we found that vomiting still remained significantly associated with deaths in severely malnourished diarrheal children (RR: 1·89, 95% CI:1.01–1.33; p=0.05). The result underscores the importance of prompt identification and management of vomiting to reduce deaths in such children.

1279

DRUG USE EVALUATION ON THE MANAGEMENT OF CHILDHOOD DIARRHEA AND USE OF ZINC SULFATE IN ETHIOPIA

Asrat Abate, Fikreslassie Alemu, Dagne Bililigne, Belete Ayalneh, Gulilat Teshome, Edmealem Ejigu, Tesfaye Seifu, Abera Alemu, Abebe Berhanu, Mihret Abreham

GHSC-PSM, Addis Ababa, Ethiopia

Diarrhea remains a leading cause of death for children under age five in Ethiopia. The WHO recommends oral rehydration salts (ORS) and zinc as the first-line treatment for childhood diarrhea; however, prescription and use of zinc for managing childhood diarrhea has been limited. To improve zinc prescribing and use in Ethiopia, the USAID Global Health Supply Chain Program-Procurement and Supply Management (GHSC-PSM) project conducted a drug use evaluation (DUE) at Addis Ketema Health Center in Dire Dawa, Ethiopia in June 2019. Using DUE findings, intervention strategies were designed and implemented through February 2020. Strategies included disseminating DUE findings, standard treatment guidelines and relevant drug formularies; socializing prescribing practices and promoting co-packaged ORS and zinc; and supportive supervision for facility staff. Advocacy at the regional health bureau resulted in the bureau's enforcement of the updated prescribing practices. A post-intervention DUE was carried out in March 2020. During each DUE, data from 100 child medical records were collected and reviewed using a tool that reflected the standard treatment guidelines and DUE techniques. The initial DUE indicated that providers had limited knowledge of zinc's benefits and how to prescribe zinc appropriately according to standard treatment guidelines. After the intervention: 1-Percentage of patients treated with zinc increased from 23% to 97% 2-Accurate dosage improved from 52% to 98% 3-Correctly prescribed regimen duration increased from 35% to 90% 4-Prescription of antibiotics decreased from 94% to 48% (indicating an empirical reduction in treatment of diarrhea with antibiotics) 5-Consumption of co-packaged ORS and zinc increased from 0% to 87%. Ensuring availability of standard treatment guidelines, providing supportive supervision, securing government enforcement of prescribing practices, and promoting use of co-packaged ORS and zinc were effective strategies to improve proper management of childhood diarrhea in Dire Dawa and present opportunities for scale-up in additional facilities in Ethiopia.

1280

SUBCLINICAL ENTERIC INFECTIONS ARE WIDESPREAD IN APPARENTLY HEALTHY INFANTS IN RURAL BANGLADESH

Fatema-Tuz Johura[1], Jarin Tasnim[1], Sahitya Ranjan Biswas[1], Riajul Islam[1], Talal Hossain[1], Kazi Sumaita Nahar[1], Rehnuma Binte Kabir[1], Indrajit Barmon[1], Susmita Basak[1], Farzana Hossain[1], Wali Ullah[1], Marzia Sultana[1], Hafizur Rahman[2], Saijuddin Shaikh[2], Hasmot Ali[2], Subhra Chakraborty[3], Alain Labrique[3], Tahmeed Ahmed[1], Md. Iqbal Hossain[1], Munirul Alam[1], Amanda C. Palmer[3]

[1]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh,* [2]*The JiVitA Project, Gaibandha, Bangladesh,* [3]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States*

Acute diarrheal disease is a major public health problem. It is the second most common cause of death worldwide in children under five years of age and contributes to poor growth. Subclinical enteric infections, independent of diarrhea, can also cause physiological and structural alterations of the gut, with adverse consequences for nutrition and growth. We investigated the prevalence of subclinical enteric infections in non-diarrheal stool samples from 6-mo-old infants (n=404) from Gaibandha, a rural setting in northwest of Bangladesh. We used Real-Time Polymerase Chain Reaction (qPCR) to detect the presence of nine genes from six pathogens: *lt, stp* and, *sth* of Enterotoxigenic *E. coli* (ETEC); *aggR* and *astA* of Enteroaggregative *E. coli* (EAEC); *eae* of Enteropathogenic *E. coli* (EPEC); *ipaH* of *Shigella spp.*; *owp* of *Cryptosporidium parvum/hominis* and *cadF* of *Campylobacter spp*. We detected *lt, stp, sth, aggR, astA, eae, ipaH, owp*, and *cadF* genes in 26.5%, 4.9%, 4.2%, 64.6%, 69.0%, 35.6%, 13.6%, 3.2% and 20.5% of stool samples, respectively. Of the samples tested, 24.3% did not carry any pathogens. EAEC infection was most common, with an overall prevalence of 73.5%; 20.3% of samples contained only EAEC. Co-infections were also common: 16.1%were co-infected with ETEC, EAEC, and EPEC. ETEC/EAEC/EPEC/*Campylobacter* co-infection was observed in 6.9% of samples. The prevalence of *Shigella-Campylobacter* co-infection was 4.9%, 3.9% and 1.7% of samples had co-infections of ETEC-EAEC-EPEC-*Shigella* and ETEC-EAEC-EPEC-*Shigella-Campylobacter*, respectively. Subclinical enteric infections, independent of diarrhea, were widespread in infants in this setting. The role of repeated illness and the potential impact of frequent subclinical infections with diarrheal pathogens present a new challenge. Interventions will depend on enhanced understanding of causal pathways, pathogenesis, and sequelae of these infections, with or without symptomatic diarrhea.

1281

ASSOCIATION BETWEEN THE GUT MICROBIOTA AT THE TIME OF ORAL CHOLERA VACCINATION AND DEVELOPMENT OF *VIBRIO CHOLERAE*-SPECIFIC LONG-TERM MEMORY B CELLS

Denise Chac[1], Fahima Chowdhury[2], Taufiqur R. Bhuiyan[2], Ashraful I. Khan[2], Regina LaRocque[3], Stephen Calderwood[3], Edward Ryan[3], Jason Harris[3], Ana Weil[1], Firdausi Qadri[2]

[1]*University of Washington, Seattle, WA, United States,* [2]*International Centre for Diarrheal Disease Research, Dhaka, Bangladesh,* [3]*Massachusetts General Hospital, Boston, MA, United States*

Cholera is an ancient cause of severe diarrhea that continues to be a major public health concern. Oral cholera vaccines (OCVs) are increasingly used in outbreaks, and immune responses to vaccination vary for reasons that are not known. The gut microbiota is an underexplored host factor that may impact memory B cell (MBC) responses to oral cholera vaccines. In this study, we compare the gut microbiome to MBC responses after vaccination with a bivalent, killed whole-cell OCV (Shanchol) in single or double dose in Bangladesh. At the time of vaccination, the baseline microbiota was assessed using 16s rRNA sequencing of fecal samples. The MBC response was quantified by calculating the area under the curve of the IgA-secreting LPS-specific MBC counts using ELISPOT over one year of follow up. Vaccinated persons were categorized as responders if LPS-specific MBC IgA responses were detected above baseline during the follow up period, and had an area under the curve of LPS-specific IgA

responses > 0.0005. Over half of vaccine recipients were responders in the single dose arm (n=11/19, 58%), double dose arm at 14 days (n=14/26, 54%), and double dose arm at 30 days (n=20/39, 51%). Overall, the population had high levels of Firmicutes (78-95% total abundance) and low levels of Bacteroidetes (12-21% total abundance). Responders in the three vaccine arms trended towards lower levels of bacteria from the class Gammaproteobacteria, which reached significance in the single dose arm ($p<0.01$, unpaired t-test). In this study, we demonstrate that the gut microbiota measured at the time of vaccination may be associated with differences in immune responses to OCVs. These findings may increase our understanding of the causes of variation in protective immunity after vaccination.

1283

EVALUATION OF THREE RAPID DIAGNOSTIC TESTS IN FIELD SETTINGS DURING CHOLERA EPIDEMIC IN CAMEROON

Yap Boum

Epicentre, Yaoundé, Cameroon

Cholera remains a major public health concern affecting 2.8 millions of cases yearly. In Cameroon, the ongoing Cholera epidemic has caused about 2335 cases and 117 deaths. Presently, PCR are the reference diagnosis methods for Cholera, but they require sophisticated laboratory infrastructure which is not always available in most affected areas. Several RDTs have been developed for cholera, but few have been tested in the settings and areas for which they were intended, with fresh stools. In this study, we aim to evaluate the performance of 3 RDTs performed by health care workers in field settings, compared to PCR and culture. We also aim to evaluate RDTs performance after alkaline peptone water enrichment, which could improve tests performance. This prospective diagnosis study started in November 2019 during the Cholera epidemic in Cameroon an is still ongoing. The study was conducted in health facilities in the North, Far North, and Littoral regions. All consenting patients with severe aqueous diarrhea, having a diarrhea episode at the study sites were recruited. Their stools were collected for RDTs, Culture, and PCR analysis. 3 brands of RDTs were evaluated in this study: Crystal Vc O1, SD Bioline Cholera Ag O1/ O139, and Cholkit. The 3 RDTs were performed in 3 rounds of testing, for a total of nine tests per sample. Results were recorded on paper questionnaires and later transferred to a data base for analysis. As of April 2020, we included a total of 330 patients, aged 6 months to 85 years old, with 43% females. Crystal Vc O1 showed a performance of (sensitivity; specificity), SD bioline (XX; XX), Cholkit (XX; XX) as compared to Culture/ PCR. Test performance was lower after enrichment with alkaline water (XX; XX). Laboratory technicians had a lower/higher performance than nurses with an overall sensitivity/specificity. Our results demonstrate that XX has the best performance and is more appropriate for Cholera field testing. It was also reported to be easier to use by the health personnel. The Lower performance after enrichment with alkaline water might be due to a non-respect of timing before or after incubation.

1284

MICROBES AND ANTIMICROBIAL RESISTANCE PROFILES AMONG PATIENTS WITH OPEN WOUNDS IN THREE HOSPITALS IN RWANDA: A CROSS-SECTIONAL SURVEY

Fredrick Kateera[1], Grace Umutesi[1], Lotta Velin[2], Moses Muwanguzi[1], Gilbert Rukundo[1], Aniceth Rucogoza[3], Carol Mugabo[1], Christian Mazimpaka[1], Jean de Dieu Gatete[1], Lisa Bebell[4], Tharcisse Mpunga[5], Robert Riviello[6], Bethany Hedt-Gauthier[7], Emil I. Mwikarago[3]

[1]*Partners in Health/ Inshuti Mu Buzima, Kigali, Rwanda,* [2]*Program in Global Surgery and Social Change, Harvard Medical School, Boston, MA, United States,* [3]*National Reference Laboratory, Rwanda Biomedical Center, Kigali, Rwanda,* [4]*Massachusetts General Hospital, Boston, MA, United States,* [5]*Butaro District Hospital, Ministry of Health, Butaro, Rwanda,* [6]*Center for Surgery and Public Health, Brigham and Women's Hospital, Boston, MA, United States,* [7]*Department of Global Health and Social Medicine, Harvard Medical School, Boston, MA, United States*

The World Health Organization's Global Action Plan on Antimicrobial Resistance (AMR) recommends countries to develop national action plans on AMR, strengthening the knowledge and evidence base through surveillance and research. However, little is known on causal agents of common infections and their sensitivity to commonly used antibiotics. We sought to characterize bacteria and AMR profiles among patients with open wounds at three rural hospitals. We conducted a cross-sectional survey among all patients with open wounds at three Rwandan Ministry of Health district hospitals (DHs) supported by Partners In Health. All eligible patients 6 months and older diagnosed with a wound infection were enrolled. Wounds were swabbed and cultured at the hospital laboratory and cultured plates were transported to the National Reference Laboratory (NRL) for pathogen identification and drug sensitivity Testing. By the end of December 2019, 233 samples had been collected. Median patient ages varied by district hospital from 23 years (IQR: 20 - 31) at the Kirehe DH to 27 years (IQR: 18 - 36) and 38 years (IQR: 24 - 53) for the Rwinkwavu and Butaro DHs, respectively. Of the 233 specimens, 166 had single growth, 49 had mixed growth, 10 samples showed no growth whilst 8 samples were determined to be contaminated. 57.6% of growth were gram-negative, including Acinetobacter spp (22.4%) and Escherichia coli (15.1%). Among gram-positive, the most common were Staphylococcus aureus (55.4%) and Staphylococcus ssp (33%). Among Staphylococcus aureus, resistance varied from 98.4% to penicillin and 72.6% to trimethoprim-sulphamethoxazole. Among gram negatives, 82.6% E. coli isolates were resistant to three antibiotics: ceftriaxone, cefepime, and ampicillin whilst 73.9% were resistant to trimethoprim-sulphamethoxazole. Acinetobacter spp isolates resistance rates were 94.6% for ceftriaxone, 83.8% to cefepime, 78.4% to trimethoprim-sulphamethoxazole and 75.7% to ciprofloxacin. The identified high AMR to commonly used antibiotics partly explain the high infection-associated morbidities, long hospital stays, high individual, and health system costs in Rwanda.

1285

THE EPIDEMIOLOGY AND CLINICAL COURSE OF INVASIVE *STAPHYLOCOCCUS AUREUS* AND GROUP A *STREPTOCOCCUS* INFECTIONS IN FIJI: A PROSPECTIVE STUDY

Li Jun Thean[1], Adam Jenney[2], Lucia Romani[3], Daniel Engelman[1], Handan Wand[3], Aalisha Sahukhan[4], Mike Kama[4], Meciusela Tuicakau[4], Joseph Kado[5], Natalie Carvalho[6], Margot Whitfeld[7], Ross Andrews[8], John Kaldor[3], Andrew C. Steer[1]

[1]*Murdoch Children's Research Institute, Melbourne, Australia,* [2]*Fiji National University, Suva, Fiji,* [3]*Kirby Institute, Sydney, Australia,* [4]*Fiji Ministry of Health and Medical Services, Suva, Fiji,* [5]*Ministry of Health and Medical Services, Suva, Fiji,* [6]*University of Melbourne, Melbourne, Australia,* [7]*University of New South Wales, Sydney, Australia,* [8]*Menzies School of Health Research, Brisbane, Australia*

Invasive *Staphylococcus aureus* (ISA) and invasive Group A *Streptococcus* (IGAS) infection cause substantial morbidity and mortality worldwide. There are however, limited data to define the epidemiology, clinical manifestations, treating practices and outcomes of these diseases particularly in resource limited settings. We conducted prospective surveillance for ISA and IGAS admissions at the referral center for the Northern Division of Fiji (population 131,914) over 48 weeks (July 2018 to June 2019). There were 52 cases of ISA and 15 cases of IGAS, corresponding to a high annual incidence of both ISA (45.2 per 100,000 person years) and IGAS (12.3 per 100,000 person years). Highest incidence was observed in people aged 65 years and older at 67.9/100,000 person years for both ISA and IGAS. ISA was more common in Indigenous Fijians compared to other ethnicities (incidence rate ratio 9.6). The most common clinical sites of infection, in order of decreasing frequency, were bloodstream, cutaneous and osteoarticular. Most cases required surgery, ISA (78.9%) and IGAS (60%). Intensive care was required in 9.6% and 13.3% of ISA and IGAS admissions respectively. Methicillin resistant *S.*

aureus was isolated in only 1.9% of ISA cases. *emm*-typing was done on all Group A Streptococcal isolates - there were 9 *emm* types, and most belonged to the E4 emm-cluster. There was a high case fatality rate observed for both ISA and IGAS infections (9.6 and 33.3% respectively). Intravenous cloxacillin was used for all cases of ISA and in 66.7% of iGAS cases. Only 46.7% of IGAS cases were prescribed intravenous penicillin. The median duration of intravenous antibiotics was 14 days for ISA and 10.5 days for IGAS. The median length of admission was 15 days for both ISA and IGAS. Patients with diabetes were 8.1 times more likely to die during admission for either ISA or IGAS compared to cases without. Fiji has a demonstrably high health burden imposed by ISA and IGAS disease. We identified demographic and comorbid risk factors associated with an increased risk of disease and fatality. Further exploration into public health prevention strategies is required to alleviate this burden.

1286

APPLYING NEXT-GENERATION SEQUENCING FOR GENOMIC ANALYSIS OF *STREPTOCOCCUS SUIS* ISOLATED IN PATIENTS WITH MENINGITIS FROM NORTHERN VIETNAM

Ha The Doan[1], Hung Manh Than[2], Hoa Mai Tran[3], Hoa Thi Nguyen[2], Rogier van Doorn[4], Bich Thi Vu[4], Hoi Thi Le[2], Trung Vu Nguyen[3]

[1]*National Taiwan University, Taipei, Taiwan,* [2]*National Hospital for Tropical Diseases, Hanoi, Viet Nam,* [3]*Hanoi Medical University, Hanoi, Viet Nam,* [4]*Oxford University Clinical Research Unit, Hanoi, Viet Nam*

Streptococcus suis is a major swine pathogen responsible for a wide array of infections including life-threatening such as meningitis, septicaemia and pneumoniae. Human *S. suis* infection has become one of the emerging infectious diseases around the world recently, especially Vietnam. *S. suis* is the most common cause of adult meningitis (33.6%) in Vietnam with a high mortality. Previous studies in Vietnam on *S. suis* have not mentioned the pathogenic mechanisms, including genomic analysis. This is one of the first studies discovering *S. suis* genomic structure and key genetic information with the level of severity in patients with meningitis in Vietnam. We sequenced 19 *S. suis* strains isolated from blood or cerebrospinal fluid of three groups including: only meningitis, meningitis and sepsis without shock, meningitis with shock. These strains were sequenced with Illumina Miseq sequencing technology at National Hospital for Tropical Diseases. Raw reads were de novo assembled using SPAdes to generate draft contigs. Sequencing typing (ST), serotype and virulence markers detection were carried out using MLST and Abricate. Phylogenetic tree of the strains was constructed using Iqtree. We used RAST service to gain some genomic information of *S. suis*. All strains in the study belonged to serotype 2, but two had capsular polysaccharide gene structure differently from the others. The dominant ST was ST1 (84%), and following by ST665 (16%). The strains with the same ST had a close relationship in the tree. Three main virulence-associated markers including suilysin, muramidase-released protein and extracellular factor were found in all strains. The genome size was about 2 million base pairs (bps) and the number of protein coding region ranged from 2036 to 2235 with approximately 70% biological function. The number of the core genomes varied from 1998 to 1890. The average GC content of strains was from 41.0% to 41.2%. Using the genomic information of *S. suis* obtained in this study will enhance the development and application of diagnostic methods and vaccine research.

1287

A SYSTEMATIC REVIEW ON ANTIMICROBIAL RESISTANCE AMONG *SALMONELLA* TYPHI WORLDWIDE

Christian S. Marchello, Samuel D. Carr, John A. Crump

University of Otago, Dunedin, New Zealand

Understanding patterns and trends of antimicrobial resistance (AMR) in *Salmonella* Typhi can guide empiric treatment recommendations and contribute to country decisions about typhoid conjugate vaccine (TCV) introduction. We systematically reviewed PubMed and Web of Science for articles reporting the proportion of *Salmonella* Typhi isolates resistant to individual antimicrobials worldwide from any time period. Isolates resistant to chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole were classified as multidrug-resistant (MDR) and isolates that were MDR plus resistant to a fluoroquinolone and a third-generation cephalosporin were extensively drug-resistant (XDR). Among the 198 articles eligible for analysis, a total of 55,459 (median 80; range 2 to 5,191 per study) *Salmonella* Typhi isolates were tested for AMR. Of isolates from 2015 through 2018 in Asia, 1,638 (32.6%) of 5,032 were MDR, 167 (5.7%) of 2,914 were resistant to a third-generation cephalosporin, and 148 (8.3%) of 1,777 to azithromycin. Two studies from Pakistan reported 14 (2.6%) of 546 isolates were XDR. In Africa, the median proportion of *Salmonella* Typhi isolates that were MDR increased each consecutive decade from 1990-1999. *Salmonella* Typhi has developed resistance to an increasing number of antimicrobial classes in Asia, where XDR *Salmonella* Typhi is now a major threat, while MDR has expanded in Africa. We suggest that continued and increased surveillance is warranted to inform empiric treatment decisions and that AMR data be incorporated into country decisions on TCV introduction.

1288

MULTI DRUG RESISTANT NONTYPHOIDAL SALMONELLA AS CAUSE OF CHILD DEATH: USING INNOVATIVE POST-MORTEM SAMPLING

Tseyon Tesfaye[1], Mulu Berihun[1], Hiwot Yizgaw[1], Mussie Berhane[1], Mersan Deresa[1], Joe Oundo[2], Nega Assefa[1], Lola Madrid[2]

[1]*College of Health and Medical Sciences, Haramaya University, Harar, Ethiopia,* [2]*London School of Hygiene & Tropical Medicine, London, United Kingdom*

Although its role in nosocomial infections is not well known, some studies show outbreaks of hospital-acquired nontyphoidal salmonella (NTS). NTS is considered cause of bacteremia and meningitis in African children. However, the role of NTS as cause of death (CoD) is unknown due to limitations in current methods to infer CoD. Child Health and Mortality Prevention Surveillance (CHAMPS) aims to determine CoD among under five children (U5) using Minimally Invasive Tissue Sampling (MITS) and advanced lab technology. Mortality surveillance was established in a demographic surveillance system (DSS) area in Eastern Ethiopia in February 2019. A death notifications system was implemented to detect MITS-eligible cases (stillbirths and U5 dead within the last 24h, belonging to DSS). Samples were analysed using conventional and molecular microbiological investigations and histopathological examination. A panel of experts assigned the final CoD after analysing demographic and clinical information, lab results and verbal autopsy. From 4th February 2019 to 3rd February 2020, 59 (59.6%) among 99 MITS-eligible cases approached, consented for MITS. Of these, CoD assignment was completed for 53; 24 stillbirths, 16 neonates and 13 U5 infants/children. Twenty-three among the 29 out of stillbirths had an infectious disease as underlying or immediate CoD. The most common pathogens were *Klebsiella pneumoniae* (n=5), *Streptococcus pneumoniae* (n=4) and *NTS* (n=3). The NTS-related deaths all occurred at Hiwot Fana Hospital, including two neonates who stayed in the Neonatal Intensive Care Unit >72h during the same period and an infant admitted at pediatrics ward for 2 weeks. All NTS isolates were resistant to first and second line antimicrobials, including third generation cephalosporins. Mortality surveillance identified NTS-related deaths that were part of a hospital-acquired multidrug resistant NTS outbreak. NTS is likely emerging as a major cause of morbidity or mortality among African children. Strategies such as improving infection prevention measures and the vaccine currently under development are important to address this lethal pathogen.

1289

CONTRIBUTION OF MICROBIAL CULTURE AND TAQMAN ARRAY CARD (TAC) ASSAY FOR DETECTING PATHOGENS IN STILLBIRTHS AND POST-MORTEM UNDER-5 BLOOD SPECIMENS IN BANGLADESH

M Ishrat Jahan[1], Mustafizur Rahman[1], Dilruba Ahmed[1], Afruna Rahman[1], Shafina Jahan[1], Sanwarul Bari[1], Farzana Islam[1], Dianna M. Blau[2], Emily S. Gurley[3], Kyu Han Lee[3], Shams El Arifeen[1], Muntasir Alam[1]

[1]*icddr,b, Dhaka, Bangladesh,* [2]*Centers for Disease Control and Prevention (CDC), Atlanta, GA, United States,* [3]*Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States*

One fifth of all child deaths globally are attributed to infections. Studies investigating systemic infections use microbial culture and/or PCR based techniques to detect specific syndrome associated infectious agents. Child Health and Mortality Prevention Surveillance (CHAMPS) project implements both microbial culture and a Taqman Array Card (TAC) based real-time PCR platform to identify diverse syndromic panels of pathogens relevant to the geographical locations and age groups. In Bangladesh, from August 2017 - August 2019, a total of 113 post-mortem blood specimens were collected from 52 stillbirths and under-5 population including 58 neonates, 2 infants and 1 child. Specimens were tested by microbial culture and TAC. The aim of this analysis was to observe the contribution of these two methods to identify infectious agents from post-mortem blood specimens. Infectious agents were detected in 54 (48%) cases either by microbial culture or TAC. Microbial culture detected infectious agents from blood in 52 cases and TAC in 13. Of the organisms detected, seven were targets in the TAC panel: *Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecium, Enterococcus faecalis* and *Escherichia coli*. *K. pneumoniae* was frequently detected (culture/TAC = 10/5) followed by *E. coli* (culture/TAC = 7/2), *A. baumannii* (culture/TAC= 3/1) and *S. aureus* (culture/TAC = 3/1). For TAC and culture, concordances were observed for *K. pneumoniae* (4/11), *E. coli* (2/7) and *A. baumannii* (1/3). Interestingly, from stillbirth cases 10 bacteria were isolated by culture but were undetected through TAC. Among these, 6 (60% of 10 bacteria) were detected from macerated type stillbirth cases indicating influence of specimen quality on molecular diagnosis. Unculturable bacteria *Rickettsia* spp. (1 stillbirth and 1 early neonate) and viruses including measles and cytomegalovirus (n=2) were identified by TAC. Our analysis indicates specimen quality and source may influence TAC. Therefore, both methods are important to determine microbial profile associated with post-mortem specimens.

1290

PREDICTING NEONATAL SEPSIS FROM MATERNAL AND INFANT CHARACTERISTICS, SIGNS, AND SYMPTOMS IN ETHIOPIA

Ronke Olowojesiku[1], Mahlet Abayneh[2], Gesit Metaferia[2], Semaria Solomon[2], Delayehu Bekele[2], Grace J. Chan[3]

[1]*AU/UGA Medical Partnership at the Medical College of Georgia, Athens, GA, United States,* [2]*St. Paul's Hospital Millenium Medical College, Addis Ababa, Ethiopia,* [3]*Harvard T.H. Chan School of Public Health, Boston, MA, United States*

Neonatal mortality accounts for nearly half of all global under-five mortality. Like many LMIC countries, the neonatal mortality rate in Ethiopia is high (28.9 per 1000 live births). A major cause of neonatal mortality in Ethiopia includes neonatal sepsis. With limited laboratory capacity, the diagnosis of neonatal sepsis is challenging and often relies on clinical criteria such as the WHO seven signs and symptoms for possible serious bacterial infection (PSBI). We identify infant and maternal characteristics associated with laboratory confirmed neonatal sepsis and mortality to develop a prediction model for neonatal sepsis in Ethiopia. For the analysis, we use data from the BARNARDS-Ethiopia study, a prospective cohort of 4583 pregnant women and their newborns in St. Paul's Hospital, Addis Ababa. We assess findings from mothers and infants by bivariate logistic regression and generate a model for prediction using Lasso regression. For model building, we randomly allocate data into a training (67%) and estimation set (33%). Over the study period, 128 (2.8%) infants had culture-confirmed sepsis. Among those with sepsis, 28 (21.9%) died after 60-day follow-up. The most prevalent maternal risk factor for sepsis is mother reports being ill (10.9%). No one maternal risk factor is noted to have a significant increased odd of sepsis. The three most prevalent observed infant findings were difficulty feeding (55.5%), elevated respiratory rate (53.3%), and chest indrawing (21.1%). Infants who are reported ill on medical interview or who have reported difficulty feeding have an increased odds of sepsis (OR 4.15, 95% CI 1.54-11.2; OR 1.54, 95% CI 1.00-2.37 respectively). A predication model with variables "infants reported ill on medical interview" and the "presence of any maternal risk factor" did not perform significantly better than current standard criteria. Until we have affordable bedside point-of-care diagnostics for bacterial sepsis, future work can be directed towards improved measures of maternal and neonatal risk factors and clinical signs/symptoms to develop a robust clinical prediction rule for neonatal sepsis in a LMIC setting.

1291

PATTERNS OF ANTIMICROBIAL RESISTANCE AMONG GRAM-NEGATIVE ISOLATES FROM NEONATES WITH SEPSIS IN ETHIOPIA

Semaria Solomon[1], Oluwasefunmi Akeju[2], Oludare A. Odumade[3], Rozina Ambachew[1], Zenebe G. Yohannes[1], Kimi Van Wickle[2], Mahlet Abayneh[1], Gesit Metaferia[1], Jordan Arvayo[2], Frederick Goddard[2], Delayehu Bekele[1], Grace J. Chan[2]

[1]*St. Paul's Hospital Millenium Medical College, Addis Ababa, Ethiopia,* [2]*Harvard T.H. Chan School of Public Health, Boston, MA, United States,* [3]*Boston Children's Hospital, Boston, MA, United States*

Newborn sepsis accounts for more than a third of neonatal deaths globally. The first-line treatment recommended by WHO is the combination of intravenous or intramuscular gentamicin with ampicillin or benzylpenicillin. Cephalosporins are the preferred alternatives when resistance to first line antibiotics are suspected. Gram-negative bacteria (GNB), the most predominant class of pathogens responsible for neonatal sepsis, readily acquire resistance to drugs that were previously effective. Our goal was to describe the patterns of antibiotic resistance among GNB at St. Paul's Hospital, a tertiary care hospital in Ethiopia. As part of the BARNARDS-Ethiopia study, we enrolled a cohort of 5216 mother-newborns pairs (in-born and out-born) between March 2017 and February 2018. For this analysis, we used data from mother-newborns collected from time of labor through 60 days of life. Among newborns with clinical signs of sepsis, we obtained blood cultures to test for bacterial and fungal growth. We identified GNB isolates from 121 neonates. Antimicrobial sensitivity testing was conducted using the dilution method. We classified phenotypes of isolates as Resistant, Intermediate or Susceptible using the Clinical & Laboratory Standards Institute cut-off for Minimum Inhibitory Concentration, the lowest concentration of an antibiotic that inhibits the bacteria's growth. For each isolate, we described the prevalence of resistance to 20 antibiotics. Of the 9 isolate types identified, the most prevalent was Klebsiella pneumonia 78% (n=94) followed by E. coli 9% (n=11). Of 121 GNB isolates, resistance to Ampicillin was found in 93% (n=113), Ceftriaxone 87% (n=105), Cefotaxime 86% (n=104), Gentamicin 83% (n=101), Ampicillin + Gentamicin 81% (n=98), Imipenem 0.8% (n=1). There was a high resistance to first-line antibiotics and third generation cephalosporins. There was less resistance to carbapenems. These results highlight the need to review the current first-line therapy for neonatal sepsis and better understand the cause and risk factors for AMR.

1292

PREVALENCE, RISK FACTORS, AND OUTCOMES AMONG PATIENTS WITH CARBAPENEM-RESISTANT GRAM-NEGATIVE BACTERIAL INFECTIONS IN GALLE, SRI LANKA

John Bollinger[1], Champica Bodinayake[2], Ajith Nagahawatte[2], Anusha Ekanayaka[2], Heshan S. Premalal[2], Gaya Wijayaratne[2], Bhagya Piyasiri[3], Madureka Premamali[2], Ruvini Kurukulasooriya[2], Bradly Nicholson[1], Christopher Woods[1], L Gayani Tillekeratne[1]

[1]*Duke University, Durham, NC, United States,* [2]*Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka,* [3]*Teaching Hospital Karapitiya, Galle, Sri Lanka*

Antimicrobial resistance (AMR) is a major public health problem that puts global health and security at risk. Carbapenem-resistant gram-negative bacteria (GNB) in particular are a growing threat, as they are resistant to almost all available antibiotics. The prevalence of carbapenem-resistant GNB is rising in many low- or middle-income countries, but comprehensive data are limited. The purpose of this study was to describe the prevalence, risk factors, and outcomes among patients with carbapenem-resistant GNB infections in Sri Lanka. The study was conducted among inpatients at the largest tertiary care hospital in the Southern Province of Sri Lanka. Laboratory-based surveillance was performed from September 2019 to January 2020 using routine clinical samples received by the clinical microbiology laboratory to identify carbapenem-resistant GNB. Prevalence of carbapenem resistance was calculated, and among a subset from whom demographic and clinical information could be obtained, risk factors such as age and previous antibiotic use were assessed for association with carbapenem resistance by performing logistic regression. From a total of 474 GNB isolates screened, 28.3% of *Enterobacteriaceae*, 21.7% of *Pseudomonas* spp., and 66.7% of *Acinetobacter* spp. exhibited carbapenem resistance. Most of these isolates came from pus (29.5%) or sterile fluid (31.3%) samples. Furthermore, carbapenem-resistant isolates exhibited high levels of resistance to other antibiotics, including ceftazidime (79.6%), piperacillin tazobactam (82.4%), gentamicin (78.1%), and ciprofloxacin (83.2%). Although none of the sociodemographic or clinical factors explored was significantly associated with having a carbapenem-resistant isolate, patients with carbapenem-resistant infections had 2.41 (CI 95% [0.796, 8.274]) times higher odds of mortality. Overall, a high prevalence of carbapenem resistance was noted. These data justify the need for consistent surveillance and larger studies to explore risk factors and reasons for poor outcome among patients with carbapenem-resistant GNB infections in Sri Lanka.

1293

ALTERNATIVE INDICATORS TO MONITOR TRACHOMA ELIMINATION; DOES LONGITUDINAL CHLAMYDIA INFECTION AND ANTIBODY DATA ADD EVIDENCE TO THE UNDERSTANDING OF RESURGENCE

Mabula Kasubi[1], George Kabona[2], Alistidia Simon[3], James Kalabashanga[4], Mkocha Harran[5], Mike French[6], Upendo Mwingira[6], Sheila West[7], Diana Martin[8], Jeremiah Ngondi[6]

[1]*Muhimbili National Hospital (University Teaching Hospital), Dar Es Salaam, United Republic of Tanzania,* [2]*Iringa Regional Referral Hospital, Iringa, United Republic of Tanzania,* [3]*Ministry of Health, Community Development, Gender, Elderly and Children, Dodoma, United Republic of Tanzania,* [4]*Muhimbili National Hosital (University Teaching Hospital), Dar Es Salaam, United Republic of Tanzania,* [5]*Kongwa Trachoma Project, Dodoma, United Republic of Tanzania,* [6]*RTI International, Washington DC, MD, United States,* [7]*Dana Center for Preventive Ophthalmology, Johns Hopkins University, Baltimore, MD, United States,* [8]*Division of Parasitic Diseases and Malaria, Centre for Disease Control and Prevention, Atlanta, GA, United States*

Trachoma is a public health problem in Mpwapwa district, Tanzania. A recent trachoma surveillance survey done three years after stopping mass drug administration (MDA) with azithromycin suggested there was a resurgence of active disease. In settings where prevalence of trachomatous inflammation - follicular (TF) is <5% and Chlamydia infection is <2% evidence suggests that resurgence of active disease is unlikely to occur. Recent studies suggest that tests for chlamydial antigen can inform interruption of transmission of *Chlamydia trachomatis.* Mathematical models suggest that TF prevalence of <5% approximates to 7% Chlamydia antibody sero-positivity. We aimed to investigate novel strategies for evaluating the resurgence of trachoma by testing markers for *C. trachomatis* infection and pgp3 antibodies. A total of 1,000 children aged 1 - 9 years old who were sampled during routine trachoma survey were included in the study. Children were examined for trachoma signs using the World Health Organization simplified grading system. Ocular swab specimens were tested for Chlamydia infection using Cepheid GeneXpert PCR platform. Dried blood spot specimens were tested for antibodies to pgp3 antigen using lateral flow assay (LFA).The mean (standard deviation) age was 4.5 (2.4) and the majority (52.7%) were male. At baseline survey conducted in 2017-2018 revealed the prevalence of TF was 9.5% (95% confidence interval [CI] 9.6 - 12.2), Chlamydia infection was 4.2% and seropositivity to chlamydia antigen pgp3 was 11.8% (95% confidence interval [CI] 9.8 - 13.7). Based on TF prevalence, one additional round of MDA is required. The results of year one longitudinal monitoring of TF, C. trachomatis infection, and antibodies to pgp3 antigen are being processed and data will be presented at the conference

1294

THE TRACHOMA ELIMINATION STUDY BY FOCUSED ANTIBIOTICS (TESFA) CLUSTER-RANDOMIZED CONTROL TRIAL FOR TRACHOMA HYPERENDEMIC DISTRICTS, AMHARA, ETHIOPIA

Andrew R. Deathe[1], Begashaw Hailemariam[2], Tigist Astale[2], Eshetu Sata[2], Ambahun Chernet[2], Andrew W. Nute[1], Kimberly A. Jensen[1], Ayalew Shiferaw[2], Demelash Gessese[2], Mulat Zerihun[2], Zebene Ayele[2], Berhanu Melak[2], Christina E. Roberts[1], Girum Mereset[3], Fisseha Admassu[4], Travis C. Porco[5], Jeremy D. Keenan[5], Thomas M. Lietman[5], Zerihun Tadesse[2], E. Kelly Callahan[1], Scott D. Nash[1]

[1]*The Carter Center, Atlanta, GA, United States,* [2]*The Carter Center, Addis Ababa, Ethiopia,* [3]*Research and Technology Transfer Directorate, Amhara Public Health Institute, Bahir Dar, Ethiopia,* [4]*Department of Ophthalmology, University of Gondar, Gondar, Ethiopia,* [5]*Francis I Proctor Foundation, University of California-San Francisco, San Francisco, CA, United States*

In areas with low to moderate trachoma prevalence, annual mass drug administration (MDA) with azithromycin has been effective in controlling trachoma as part of the surgery, antibiotics, facial cleanliness, and environmental improvement (SAFE) strategy. This has not been the case in all hyperendemic regions, however. After 10+ years of community-wide MDA, the current approach in the Amhara region of Ethiopia, many districts remained hyperendemic. Previous research, from both randomized trials and modeling studies, has further suggested that annual MDA may not be sufficient to reach elimination in regions like Amhara. The Trachoma Elimination Study by Focused Antibiotics (TESFA) study is a cluster-randomized control trial that aims to identify whether an alternative, enhanced antibiotic regimen can generate greater and faster impact than routine intervention. The intervention arm will receive community-wide MDA plus an additional two rounds of MDA targeted to children aged 2-9 years in quick succession (1-2 weeks). This arm will be compared to a control arm that receives community-wide MDA. Sixty-four sub-districts will be randomly chosen, and half will be allocated to each treatment arm. MDA interventions will be conducted at the sub-district level. The primary outcome is the community-level prevalence of *Chlamydia trachomatis* (*Ct*) infection in children aged 6 months to 9 years at the 12-month follow-up. For outcome monitoring, data will be collected within one sentinel village per sub-district. To allow for a community-level analysis of *Ct* infection prevalence at each follow-up examination (baseline, months 1, 12, and 24), 50 children aged 6 months to 9 years within each sentinel village will be randomly selected. We plan to implement in 3 districts within the North Wollo zone, which has historic and persistent hyperendemic trachoma. This study was approved

by internal review boards at Emory University and at the federal level in Ethiopia. TESFA responds to the needs of trachoma programs facing persistently hyperendemic districts and may help these programs achieve elimination faster.

1295

THE USE OF PHOTOGRAPHIC GRADING FOR TRACHOMA DIAGNOSIS WITHIN TRACHOMA IMPACT SURVEYS: A PILOT STUDY IN AMHARA REGION, ETHIOPIA

Cassidy Whitson[1], Andrew W. Nute[2], Begashaw Hailemariam[3], Tigist Astale[3], Demelash Gessese[3], Eshetu Sata[3], Mulat Zerihun[3], Mahiteme Haile[4], Taye Zeru[4], Berhanu Melak[3], Zebene Ayele[3], Dionna Wittberg[5], Zerihun Tadesse[3], E. Kelly Callahan[2], Jeremy D. Keenan[5], Fisseha Admassu[6], Scott D. Nash[2]

[1]*Rollins School of Public Health, Emory University, Atlanta, GA, United States,* [2]*The Carter Center, Atlanta, GA, United States,* [3]*The Carter Center, Addis Ababa, Ethiopia,* [4]*Amhara Public Health Institute, Bahir Dar, Ethiopia,* [5]*Francis I Proctor Foundation, University of California, San Francisco, CA, United States,* [6]*Department of Ophthalmology, University of Gondar, Gondor, Ethiopia*

Photographic grading for trachoma diagnosis within programmatic surveys is tempting for trachoma control programs, particularly in elimination settings where available cases for grader training are rare. This pilot study aimed to assess the feasibility of including photographers within survey teams to take gradable photos, the effectiveness of an in-country grading center, and the comparability of field and photographic grading. During a 2017 population-based survey in Amhara, Ethiopia, certified field graders assessed trachomatous inflammation-follicular (TF), trachomatous inflammation-intense (TI), and trachomatous scarring (TS). As part of this pilot study, a trained photographer joined survey teams to take 2 photographs of each eye using a Canon EOS 60D camera with a macro lens following field grading. At the newly developed Gondar Grading Center at the University of Gondar, trained photographic graders, masked to the field grades, graded photographs for TF, TI and TS on custom-build software. Photographs were graded by 2 different graders, and a 3rd provided adjudication for discrepant grades. Field grading was completed for 1,243 individuals from 10 villages and 6.2% had TF, 6.4% had TI, and 3.8% had TS. Further, 2,478 eyes were photographed. Photographic grading took 5 days, and 1 photograph was deemed ungradable. Inter-rater agreement between the 2 photographic graders for TF was percent agreement (PA): 96.5%, Cohen's kappa coefficient (κ)=0.69, 95% Confidence Interval (CI): 0.66-0.75; for TI was PA: 94.6%, κ=0.41, 95%CI: 0.32-0.49; and for TS was PA: 85.2%, κ=0.53, 95%CI: 0.49-0.58. After initial photo grading, adjudication was needed for 94 TF, 143 TI, and 370 TS discrepant photos. After adjudication, the agreement between field and photo grades for TF was PA: 96.7%, κ=0.70, 95%CI: 0.64-0.76; for TI was PA: 94.7%, κ=0.32, 95%CI: 0.23-0.41; and for TS was PA: 83.5%, κ=0.23, 95%CI:0.19-0.27. Gradable photographs can be taken within surveys, can be graded quickly in-country, and can provide TF diagnoses comparable to field grading. With standardization, photographic grading may be possible for programmatic use.

1296

RESULTS OF TRACHOMA SURVEILLANCE SURVEYS IN 21 HEALTH DISTRICTS OF THE FAR NORTH AND THE NORTH REGIONS OF CAMEROON AT LEAST TWO YEARS AFTER STOPPING MASS DRUG ADMINISTRATION

Emilienne Epée[1], **Bella Assumpta**[1], Georges Nko'Ayissi[1], Julie Akame[2], Patrick Mbia[2], Carine Fokam[2], Benoit Dembele[3], Jennifer Magalong[4], Ismael Teta[2], Steven D. Reid[5], Yaobi Zhang[3]

[1]*Ministry of Public Health, Yaoundé, Cameroon,* [2]*Helen Keller International, Yaoundé, Cameroon,* [3]*Helen Keller International, Regional Office for Africa, Dakar, Senegal,* [4]*Family Health International 360, Washington, DC, United States,* [5]*Helen Keller International, New York, NY, United States*

Trachoma was endemic and required mass drug administration in 18 of 30 health districts (HDs) of the Far North region and in three of 15 HDs of the North region of Cameroon. Each of these HDs received 1-5 rounds of mass drug administrations (MDA). From 2014-2017 all these HDs progressively stopped MDA as they successfully completed their trachoma impact surveys (TIS). In 2019, these HDs underwent a trachoma surveillance survey (TSS) in a total of 18 evaluation unit (EU) at least two years after stopping MDA, according to recommendations from the World Health Organization (WHO). This was a descriptive cross-sectional survey based on a random cluster sampling recommended by WHO. 24 clusters per EU in the North region and 27 clusters per EU in the Far North region were selected respectively, and 30 households per cluster were visited. Teams of surveyors (one grader and one recorder per team) were trained on the Tropical Data-developed training modules before beginning field data collection. Graders utilized the WHO simplified grading systems; recorders used smartphones to enter and send data via the Tropical Data platform. In total, the teams examined 20,995 children ages 1-9 years from 477 clusters in 18 EUs. The results showed that 16/18 EUs reported TF prevalence, ranging 0.06% (95% confidence interval: 0%-0.22%) to 3.66% (95% CI: 2.6%-4.6%), below 5%, the prevalence threshold for elimination of trachoma as a public health problem. However, two EUs, representing Goulfey and Makary HDs, reported 6.91% (95% CI: 5.4%-8.4%) and 10% (95% CI: 8.4%-11.5%) TF prevalence respectively. This survey revealed that the TF prevalence of 19 HDs from 16 EUs maintained the trachoma elimination threshold at least two years after the TIS. The Ministry of Health plans to investigate further the reasons of these TSS failures in two EUs prior to restarting MDAs.

1297

DISTRICT-LEVEL CORRELATES OF OCULAR *CHLAMYDIA TRACHOMATIS* INFECTION AMONG CHILDREN AGED 1-5 YEARS AFTER 5 YEARS OF THE SAFE STRATEGY IN AMHARA, ETHIOPIA

Andrew W. Nute[1], Ambahun Chernet[2], Mulat Zerihun[2], Eshetu Sata[2], Tigist Astale[2], Demelash Gessese[2], Gedefaw Ayenew[2], Berhanu Melak[2], Zebene Ayele[2], Mahiteme Haile[3], Taye Zeru[3], Zerihun Tadesse[2], E. Kelly Callahan[1], Scott D. Nash[1]

[1]*The Carter Center, Atlanta, GA, United States,* [2]*The Carter Center, Addis Ababa, Ethiopia,* [3]*Amhara Public Health Institute, Bahir Dar, Ethiopia*

Trachoma control programs make decisions regarding mass drug administration (MDA) based on the district prevalence of trachomatous inflammation-follicular (TF) among children aged 1-9 years. Once MDA programs begin however, it has been demonstrated that TF prevalence consistently overestimates the prevalence of *Chlamydia trachomatis* (*Ct*), the causative agent of trachoma. Understanding district-level correlates of *Ct* infection post-MDA could help programs better target interventions. Between 2011-2015, the Trachoma Control Program in Amhara, Ethiopia conducted 150 district-level surveys to estimate the prevalence of TF and trachomatous inflammation-intense (TI) among children aged 1-9 years, the *Ct* prevalence among children aged 1-5 years, as well as household water, sanitation, and hygiene (WASH) indicators throughout Amhara. Ocular swabs were collected from 15,632 children and assayed using the Abbott m2000 assay. Logistic regression was used to analyze associations

between trachoma signs and WASH indicators with district presence of infection. Linear regression was used to analyze associations with *Ct* prevalence among those districts with infection. A total of 86/150 (57.3%) districts had infection detected, with resulting district prevalence estimates ranging between 0.5-38.3%. Associations were observed between a district presence of *Ct* infection and both TF (odds ratio [OR]: 1.2, 95% confidence interval (CI): 1.13-1.26) and TI (OR: 2.24, 95%CI: 1.73-2.90). WASH indicators such as latrine coverage, presence of a hand washing station, and an improved water source were associated with either the presence of *Ct* infection or infection prevalence. The prevalence of clean face among children was statistically significant in both logistic (OR: 0.92, 95%CI: 0.90-0.97) and linear (β: -0.23, 95%CI: -0.41 - -0.05) models. WASH indicators were correlated with *Ct* infection in this post-MDA setting, suggesting that trachoma control programs serving hyperendemic areas should continue to build strong partnerships towards collaboration and implementation of WASH improvements with education, water and sanitation sectors.

1298

TRACHOMA TRANSMISSION PATHWAYS: A QUALITATIVE STUDY OF FACTORS RELATED TO INCREASED ACTIVE TRACHOMA IN MOROTO, NEBBI AND BULIISA DISTRICTS OF UGANDA

Martin K. Osilo[1], Ben Binagwa[1], Stella Agunyo[1], Mike Mukirane[1], Betty Nabatte[1], Francis Mugume[2], Gilbert Baayenda[2], Esther Nkolo[3], Elizabeth Plunkett[4], Elizabeth Fitch[5], Lauren Brown[4], Crowley Kathryn[4], J Ngondi c/o M. Ngondi[4]

[1]*RTI International, Kampala, Uganda,* [2]*Ministry of Health, Kampala, Uganda,* [3]*WI-HER, Kampala, Uganda,* [4]*RTI International, Washington, DC, United States,* [5]*RTI International, RTP, NC, United States*

Implementation of the WHO surgery, antibiotics, facial cleanliness and environmental improvement (SAFE) strategy in Uganda started in 2007. Following nation-wide program scale-up and attainment of elimination targets of trachomatous inflammation-follicular (TF) of <5% in most districts, the Ministry of Heath set an initial target for national-wide elimination of trachoma by the year 2021. However, impact surveys in Moroto showed that TF prevalence had increased from 14.6% in 2016 to 16.2% in 2019. In addition, surveillance surveys in Buliisa and Nebbi in 2019 showed that TF prevalence had recrudescence above the 5% elimination threshold (i.e. 5.8% and 7.8%, respectively). We present findings of a qualitative study of factors related to the increase of TF prevalence. We used key informant interviews with district leaders and partners, and focus group discussions with community members in the three districts. Data was summarized by themes based on the data collection tools using a daily interview summary sheet. Thematic analysis was done by district to synthesize evidence to inform future improvements of mass drug administration (MDA) and broader SAFE implementation. The findings suggest that trachoma remains a public health problem in the three districts due to a combination of social, environmental and programmatic factors. Social factors were absenteeism during MDA, poor facial hygiene, cross-border nomadic migration, and low risk perception among community members. Environmental factors were poor household living conditions, and low access to water, sanitation and hygiene (WASH) facilities. Programmatic factors were inadequate district capacity to monitor trachoma, inadequate interventions targeting nomadic communities, and limited interventions for WASH. These findings are important to consider when planning future implementation of SAFE. As these factors may be district or community specific, it is important to create context specific plans to address the changes needed to enhance trachoma elimination in these three districts in Uganda.

1299

STOPPING MASS DRUG ADMINISTRATION FOR TRACHOMA IN 15 HEALTH DISTRICTS IN GUINEA

Fatoumata Sakho[1], André Géopogui[1], **Christelly Badila Flore**[2], Mamadou Siradiou Baldé[1], Cece Nieba[1], Lamah Lamine[2], Fatou Gueye[3], Brian Fuller[3], Daniel Cohen[4], Yaobi Zhang[5], Steven D. Reid[3]

[1]*Ministry of Health, Conakry, Guinea,* [2]*Helen Keller International, Conakry, Guinea,* [3]*Helen Keller International, New York, NY, United States,* [4]*Family Health International 360, Washington, DC, United States,* [5]*Helen Keller International, Regional Office for Africa, Dakar, Senegal*

Baseline trachoma mapping in Guinea conducted from 2011 to 2016 identified 18 endemic health districts (HD) that needed mass drug administration (MDA). Between 2014-2018, 15 of these HDs completed the required rounds of MDA. At least six months after the last treatment, impact surveys were conducted in these 15 HDs between 2017 and 2019. A cross-sectional survey using a cluster randomized sampling method was conducted to assess the prevalence of trachomatous inflammation - follicular (TF) in children aged 1-9 years old and trachomatous trichiasis (TT) in adults aged ≥15. 15 HDs were evaluated in 18 evaluation units (EU) and 20 villages and 30 households were randomly selected in each EU. TF and TT were diagnosed by clinical examination using the World Health Organization (WHO) simplified grading system. Indicators of household access to water, sanitation and hygiene (WASH) were also collected. A total of 60,727 people from 11,108 households in 360 clusters were screened (28,531 males and 32,196 females). 30,354 children aged 1-9 years and 26,479 adults were examined. The TF prevalence ranged from 0.2% (95%CI: 0.1-0.2%) to 2.1% (95%CI: 1.9-2.3%), a reduction from the baseline prevalence in the 15 HDs ranging from 6.2 (95%CI 5.1-7.5%) to 41.8 (95%CI 39.4-44.2%). The TT prevalence ranged from 0.0% (95%CI: 0.0-0.1%) to 1.7% (95%CI: 1.5-1.8%), compared with the baseline prevalence from 0.0% (95%CI: 0.0-0.2%) to 2.8% (95%CI: 2.3-3.5%) in the 15 HDs. Household-level access to improved sanitation facilities ranged by EU from 7% to 63%. Household-level access to an improved source of water for face and hand washing ranged by EU from 41% to 90%. Access to safe drinking water ranged by EU from 41% to 99%. Overall, the impact survey results showed that all 15 HDs have a TF prevalence <5%, reaching the criteria to stop MDA in these HDs. This represents approximately a population of over 4 million people living in areas no longer requiring MDA. However, WASH interventions should be continued. 11 HDs have TT prevalence <0.2% while 4 HDs still have TT prevalence >0.2%, which suggests the need to continue TT surgical intervention in Guinea.

1300

TWELVE-YEAR LONGITUDINAL TRENDS IN TRACHOMA PREVALENCE AMONG CHILDREN AGED 1 TO 9 YEARS IN 160 DISTRICTS OF AMHARA REGION, ETHIOPIA 2007 TO 2019

Eshetu Sata[1], Andrew W. Nute[2], Tigist Astale[1], Mulat Zerihun[1], Demelash Gessese[1], Zebene Ayele[1], Ambahun Chernet[1], Berhanu Melak[1], Kimberly A. Jensen[2], Mahiteme Haile[3], Taye Zeru[3], Zerihun Tadesse[1], E. Kelly Callahan[2], Jeremiah Ngondi[4], **Scott D. Nash**[2]

[1]*The Carter Center, Addis Ababa, Ethiopia,* [2]*The Carter Center, Atlanta, GA, United States,* [3]*Amhara Public Health Institute, Bahir Dar, Ethiopia,* [4]*RTI International, Washington, DC, United States*

To eliminate trachoma as a public health problem, the WHO recommends the SAFE (Surgery, Antibiotics, Facial cleanliness, and Environmental improvement) strategy. The Trachoma Control Program in the Amhara Region of Ethiopia, where all districts were trachoma endemic, began scale-up of SAFE in 2007. The Program has distributed approximately 15 million doses of antibiotic per year since scaling-up and has also provided annual village- and school-based health education and assisted in the construction of latrines throughout the region as part of the F and E components. The aim of this study was to provide an update on the prevalence of trachoma among children aged 1-9 years as of the most recent trachoma impact or surveillance survey in all 160 districts of

Amhara. The 160 most recent population-based district-level surveys were conducted between 2015-2019 and included 106,321 children aged 1-9 years examined for trachoma by certified graders. As of 2019, 45 (28%) districts were below the elimination threshold of <5% prevalence of trachomatous inflammation-follicular (TF). There was a strong relationship between the TF prevalence observed at the first impact survey and eventual achievement of TF elimination threshold. Of the 26 districts with a first impact survey <10% TF, 20 (76.9%) had <5% TF at the most recent survey. Of the 75 districts with a first survey between 10-29.9% TF, 21 (28.0%) had <5% TF at the most recent survey. Finally, among 59 districts >30% TF at first survey, 4 (6.8%) had <5% TF by 2019. As of 2019, 30 (18.8%) districts remained with TF >30%. By 2019 the trachomatous inflammation-intense prevalence was <3% in 128/160 (80.0%) districts. 27 districts had a ≥60% prevalence of household access to water within 30 minutes (district range:10.1-99.0%) and 85 districts had ≥60% prevalence of household improved water source (range:7.5-96.5%). Amhara has seen great reductions of active trachoma since the start of the program. A strong commitment to the SAFE strategy coupled with data driven enhancements and adaptations are necessary to drive elimination of trachoma as a public health problem regionally in Amhara and nationwide in Ethiopia.

1301

POST-ENDEMIC SURVEILLANCE FOR TRACHOMA: SUCCESSES AND CHALLENGES FROM A HIGHLY ENDEMIC REGION, AMHARA, ETHIOPIA

Tigist Astale[1], Demelash Gessese[1], Andrew W. Nute[2], Eshetu Sata[1], Mulat Zerihun[1], Kimberly A. Jensen[2], Mahiteme Haile[3], Taye Zeru[3], Berhanu Melak[1], Zebene Ayele[1], Gedefaw Ayenew[1], Zerihun Tadesse[1], E. Kelly Callahan[2], Scott D. Nash[2]

[1]*The Carter Center, Addis Ababa, Ethiopia,* [2]*The Carter Center, Atlanta, GA, United States,* [3]*Amhara Public Health Institute, Bahir Dar, Ethiopia*

Trachomatous inflammation—follicular (TF) prevalence of <5% among children aged 1-9 years is one of the thresholds to eliminate trachoma as a public health problem. For districts that have achieved this threshold based on impact survey results, a follow-up surveillance survey is conducted after at least 2 years after the cessation of mass drug administration (MDA) to determine whether elimination is sustained. Understanding the characteristics of districts that do not sustain TF <5% is important given restarting MDA programs is resource intensive. In Amhara, 39/165 (24%) districts have reached the TF elimination threshold and have had surveillance surveys between 2015-2019. Multistage cluster-random sampling was used to select a population-based sample. Certified graders examined survey participants for trachoma signs. Among these 39 districts, 30 (77%) remained below the TF threshold at surveillance survey. There was a strong relationship between the TF prevalence observed at the first impact survey and the prevalence at the surveillance survey. All 9 districts with a first impact survey <5% TF remained <5% at surveillance. Of the 10 districts with a first impact survey between 5-9.9% TF, 8 (80%) remained <5%, and of 20 districts with a first impact survey between 10-29.9%, 13 (65%) remained <5%. Among the 9 districts where TF was >5% at surveillance, the TF prevalence ranged from 5.1% (95% Confidence Interval [CI]: 3.2-7.4%) to 11.2% (95%CI: 4.6-18.2%). The prevalence of trachomatous inflammation-intense (TI) was ≤1% in all 9 districts. Prior evidence of *Chlamydia trachomatis* infection in a district was not predictive of TF >5% at surveillance. Furthermore, water and sanitation indicators were not markedly lower in districts with TF >5%, compared to those <5% at surveillance. Eight of the 9 (89%) districts with a surveillance survey >5% were adjacent to districts that remain trachoma endemic. Although most districts were <5% TF at surveillance in Amhara, collecting additional trachoma data such as infection and serological markers during these surveys could help determine whether a TF >5% is due to sampling variability or true resurgence.

1302

WHAT TO DO DIFFERENTLY TO ENHANCE ELIMINATION OF TRACHOMA IN TANZANIA: LESSONS LEARNED FROM DISTRICTS WITH PERSISTENT TRACHOMA FOLLICULAR (TF) PREVALENCE OF MORE THAN 5%

Gerald Robi[1], Denis Kailembo[1], Veronica Kabona[1], Oscar Kaitaba[2], George Kabona[2], Kerry Dobies[3], Mary Linehan[3], Abdel Direny[3], Jeremiah Ngondi[4], Josh West[5], Benjamin Crookston[5]

[1]*IMA World Health, Dar Es Salaam, United Republic of Tanzania,* [2]*Ministry of Health, Community Development, Gender, Elderly and Children,, Dar Es Salaam, United Republic of Tanzania,* [3]*IMA World Health, Washington, DC, United States,* [4]*RTI International, Washington, DC, United States,* [5]*Brigham Young University, Provo, UT, United States*

Tanzania has reduced the number of districts requiring mass drug administration (MDA) for Trachoma by over 90% (71 to 6). These districts have successfully passed the trachoma impact survey indicating continuing transmission cannot be sustained. Despite this progress, the Tanzania neglected tropical diseases (NTD) program must still address districts where Trachoma transmission persists. Four districts failed TIS in 2014, 2016, and 2019 while two districts failed Trachoma surveillance survey in 2019, two years after stopping MDA. After failures in 2019, the NTD program conducted key informant interviews and reviewed MDA coverage history, health-facility MDA coverage, and coverage evaluation surveys (CES) to identify possible reasons for failures and to explore appropriate actions to improve program effectiveness. The program further conducted a desk review to identify key reasons and factors that have contributed to failure. The MDA historical data from 2004 revealed that all districts have trachoma follicular (TF) baseline prevalence >20% in children of 1-9 years of age, and had at least 8 rounds of MDA though all districts reported MDA coverage ≥80% at least in two rounds (ranging 2-5). In addition, the data showed low access to water (6-28%) and sanitation (4-29%) in these areas. The four main reasons for low MDA coverage are absenteeism, wrong perception of MDA, lack of information on MDA, and lack of drug inventory. The data allows to identify some challenges during MDA implementation such as insufficient number of community drug distributors (CDDs) and MDA supervisors, and tetracycline not used in MDA for those ineligible for Zithromax. Based on desk review results, recommendations have been made to Tanzania NTD programs to promote increased access and advocate with WASH partners to improve access to facial cleanliness and environmental improvement (F&E) component of the SAFE strategy to decrease trachoma infection in these four districts. Also, the program will implement MDA, coverage supervisor tool (CST) and mop-up if needed at proper times, and will consider introducing tetracycline in MDA for those ineligible for Zithromax.

1303

NOVEL THERAPIES FOR GLOBAL HEALTH DISEASES

Paul G. Ashigbie[1], Jonathan Spector[1], Thierry T. Diagana[2]

[1]*Novartis Institute for Tropical Diseases, Cambridge, MA, United States,* [2]*Novartis Institute for Tropical Diseases, Emeryville, CA, United States*

Normal market dynamics often do not favor investments in research and development for novel therapies that address global health problems. This study aims to analyze the landscape of pharmaceutical innovations for diseases that disproportionately affect populations in low-resource settings. The medicine registration databases of major national health authorities were examined for innovative medicines approved between January 2000 and March 2020 for treatment of 20 Neglected Tropical Diseases (NTDs) and 16 other infectious illnesses identified by the Access to Medicines Foundation to be priority conditions in LMICs. Drugs in stages I-III of clinical development were identified through Clinicaltrials.gov. The focus was first-in-class medicines with a novel mechanism of action; re-purposed and follow-on drugs were excluded. Only 17 of the 36 disease areas of interest had an innovative medicine approved or in development during the study period. Eleven medicines were approved for 4 diseases; six for HIV/AIDS, three for tuberculosis, and the remainder

for malaria and cryptosporidiosis. A total of 111 medicines were in clinical development for fifteen diseases; 9% (10) of these were in phase III, 49% (54) in phase II, and 42% (47) were in phase I. Therapies in late-stage (phase III) trials were for HIV/AIDs, Ebola virus disease, malaria, human African trypanosomiasis, and Chagas disease. Four diseases (HIV/AIDS, malaria, tuberculosis, and Ebola virus disease) accounted for more than 80% of medicines in development with more than half addressing HIV/AIDS. 55% (67) of medicines (approved or in development) were small molecules, 27% (33) were monoclonal or polyclonal antibodies, and 16% (20) were gene or cell therapies. Most (76%) of the biologic medicines were for HIV/AIDS. The breadth of innovative drug discovery for global health diseases is not commensurate with the medical needs and largely centers on three diseases: HIV/AIDS, tuberculosis, and malaria. New strategies may be needed to strengthen the global research portfolio of novel medicines for populations in low resource-settings.

1304

AN UNUSUAL CLINICAL PRESENTATION OF CENTRAL AMERICAN PHONEUTRISM

Monica R. Pachar[1], Jose A. Suarez[2]

[1]*Hospital Santo Tomas, Panama, Panama,* [2]*Instituto Conmemorativo Gorgas para Estudios de la Salud, Panama, Panama*

The spiders of the *Phoneutria* genera are wandering hunters from the *Ctenidae* family. In this case, we report the first case of severe Phoneutrism in Panama with acute neurological manifestations. A 65y male from a rural area in Panama, was bitten by a spider in his arm during field labor. He described a medium size spider of gray color, it jumped to the floor and "armed" itself, lifting in the air the front legs and exposing a orange color around the fangs. Two minutes after the contact he collapsed. He was found 2 hours later and taken to a local hospital from where he was transfer to our unit. He didn't receive spider anti venom. He had mild elevated blood pressure, flaccid right hemiplegia with fasciculations in the left lower limb and some degree of general weakness. Two bite marks were evident in the upper right limb. The blood workpup was unremarkable. Simple brain ct scan in the first 12h showed an area of hipodensity in the left parietal lobe. Later angio-MRI and EEG were normal. Because of persistent weakness with new neurological findings: hyperrreflexia and bilateral clonus conduction studies were performed, and it revealed mono-neuritis multiplex. In his last visit to the ambulatory clinic, he was walking with the help of a cane. The morphological description of the spider and the neurotoxicity presented in the patient are corresponding to Phoneutria spp. The spider neurotoxins have no known direct effects on the CNS and do not cause thrombosis, but they can cause seconday effects through release of autonomic transmitters (e.g catecholamines). There is some experimental evidence that Phoneutria venom can damage the blood-brain-barrier. Our hypothesis is that the envenoming caused a surge of blood pressure which in his case probably caused transient parietal lobe ischaemia through vasospam in his cerebral microvasculature with a similar phenomenon in the spinal cord. It's remarkable the spectacular presentation of this case of Phoneutrism, and it leave us with more questions than answers. We need more research in the field of toxinology in our country to be able to give answers to our rural communities .

1305

BLASTOMYCOSIS IN KENTUCKY

Evelyn B. Villacorta Cari, Nicole E. Leedy, Julie Ribes, Jaime I. Soria, Thein Myint

University of Kentucky, Lexington, KY, United States

Blastomycosis is an endemic fungal infection caused by *Blastomyces dermatitidis*, a dimorphic fungus can cause disease in immunocompetent and non-immunocompetent patients. In this retrospective study were included patients with Blastomycosis diagnosed at the University of Kentucky Hospital from 2005 to2019, logistic regression was used to identify variables associated with severe infections. The analysis included 77 patients; among these patients, the median age was 48 years old (range: 16 - 89); 62 (80.5%) were male, 66 (85.7%) identified as white. History of u tobacco use was reported in 51 (69.8%). Coal miner 8/47 (17.0%) and construction worker 7/47 (14.8%) were the most common occupations. 25/75 (33.3%) were obese, 23 (29.8%) were diabetic, 21 (27.2%) had COPD, 25 (32.4%) had at least one immunosuppressive condition, no HIV-infected patient was found in this cohort. The most common clinical manifestations were dyspnea in 34 (44.1%) and cough in 33 (42.8%). The median duration of illness was 87 (3-365) days. 54/64 (84.3%) had a positive culture, and 45/62 (72.5%) had positive histopathology. Pulmonary blastomycosis was the most common presentation 60 (77.9%), acute pneumonia in 19 (61.2%), chronic pneumonia in 10 (32.2%), and nodular lung lesions in 34 (56.6%). Initial antifungal treatment was amphotericin B liposomal in 37/75 (49.3%) and 18/56 (32.1%) required intensive critical care. Overall mortality was 11 (14.2%). A multivariable analysis was performed to find predictors of severe blastomycosis infection, no associations were seen with factors as male sex (OR 0.46; 95%CI 0.04 - 4.44), and was confirmed that significant independent associated risk factors for severe infection were age older than 50 (OR 6.59; 95%CI 1.27-34.07), obesity (OR 9.41; 95% CI 1.67-52.9), diabetes (OR 9.29; 95% CI 1.72-49.99), and anemia (OR 9.41; 95% CI 1.56-56.55). Blastomycosis is an endemic infection in Kentucky, it may produce disseminate and severe disease with significant mortality. Older age, obesity, diabetes, and anemia at admission were associated with severe disease.

1306

BURDEN OF INTESTINAL PARASITES ARE ASSOCIATED WITH IMMUNE ACTIVATION IN PEOPLE WITH HUMAN IMMUNODEFICIENCY VIRUS

Rojelio Mejia

Baylor College of Medicine, Houston, TX, United States

There are over 2 billion gastrointestinal parasitic infections worldwide and affect people in the same regions as high HIV prevalence. These endemic regions have estimated HIV/parasitic co-infection rates of over 50%. Specifically, infections with *Cryptosporidium* (*parvum* or *hominis*) can be between 5% and 32% of HIV-infected patients with diarrhea. Despite the high prevalence of co-infections, little is known about the impact that intestinal parasite burden has on HIV-infected morbidity. A prospective study was performed on 100 people living with HIV to evaluate the impact on immune activation co-infected with intestinal pathogens. Multi-parallel real-time quantitative PCR was performed on stool for 12 intestinal parasites and pathogens include *Cryptosporidium* species*, Entamoeba histolytica, Giardia lamblia, Encephalitozoon intestinalis, Enterocytozoon bieneusi, Blastocystis hominis, Isospora belli, Ascaris lumbricoides, Ancylostoma duodenale, Necator americanus, Strongyloides stercoralis,* and *Trichuris trichiura*. A recombinant *Strongyloides* antigen ELISA was performed on serum from all participants. The enrollment of patients came from Houston, Texas, which includes Harris Health Harris Health System. Many of the patients that are cared for in the Harris Health System are uninsured. Preliminary studies show *Cryptosporidium* species, *Enterocytozoon bieneusi*, and *Strongyloides stercoralis* as the predominate infections. Individuals infected with heavy burden of *Cryptosporidium* DNA (>400 fg/µl) had an inverse relationship with low CD4+ cell count (11 cells/ml). Patients with low Cryptosporidium DNA concentrations (< 0.2 fg/µl) had higher CD4+ counts (525 cells/ml). The opposite was seen with HIV RNA viral loads, with heavy *Cryptosporidium* burden associated with high viral loads (23,000 copies/ml) compared to low *Cryptosporidium* DNA burden and low HIV viral loads (25 copies/ml). This study shows an association with parasite burden and CD4+/ HIV viral loads. Pending studies include measuring soluble CD163 and intestinal fatty acid binding protein for makers of intestinal inflammation.

1307

AETIOLOGIC DIAGNOSIS IS RARELY CONFIRMED AMONG PATIENTS WITH MENINGITIS AND ENCEPHALITIS IN SRI LANKA

Weerasinghe M. D. G. B. Wijayaratne[1], K.P.G.M.P.D. Sucharitharathne[1], Champika Bodinayake[1], Vasantha Devasiri[1], Bhagya Piyasiri[2], Ruvini Kurukulasooriya[1], Chris Woods[3], L. Gayani Tillekeratne[3], Ajith Nagahawatte[1]

[1]*University of Ruhuna, Galle, Sri Lanka,* [2]*Teaching Hospital Karapitiya, Galle, Sri Lanka,* [3]*Duke University, Durham, NC, United States*

The epidemiology of central nervous system (CNS) infections in Sri Lanka has not been fully characterized. A prospective study conducted to determine the epidemiology and aetiology of patients with meningitis or encephalitis in Sri Lanka. Consecutive patients admitted to a tertiary care center in Southern Province, Sri Lanka were screened from March-October 2019. Children and adults admitted with a clinical diagnosis of meningitis or encephalitis were enrolled. Demographic and clinical information were extracted from the medical records. We performed descriptive analyses to determine prevalence and aetiology. A total of 47,786 patients were screened during the study period, and 132 meeting criteria for meningitis/encephalitis were enrolled. Overall, 57.6% of enrolled patients were male and the age range was 1 to 89 years (mean 38.73 years, SD 27.17). Symptoms at admission included headache (58, 43.9%), confusion (41, 31.1%) and seizures (27, 20.5%). Patients had meningismus (59, 75.6%) and papilloedema (2, 2.6%) on examination. Majority (73.27%) had a Glasgow Coma Scale level of 13-15. Majority (94.7%) had a lumbar puncture, with only 6.5% performed before administration of antibiotics. Cerebrospinal fluid (CSF) gram stain and culture was negative in all patients. CSF molecular testing revealed two patients with varicella zoster virus (2/21) and one with herpes simplex virus (1/21). One patient had CSF positive for *Streptococcus pneumoniae* antigen (1/5). Of 89 blood cultures performed, 13.48% were positive for significant pathogens with most common being *S. pneumoniae* (6) and *Escherichia coli* (4). Only 12 patients had a confirmed aetiology (11.11%, 12/108) at discharge. Multiple antimicrobials were given to all patients including third generation cephalosporins (100%), acyclovir (74.2%), vancomycin (24.24%) and meropenem (15.15%). Six (4.6%) patients died. CNS infection was associated with high morbidity and mortality. The majority of CNS infections had an unknown aetiology, but all patients received multiple antimicrobials. Newer diagnostic tools are essential for the treatment of meningitis and encephalitis in Sri Lanka.

1308

ELEVATED ENVIRONMENTAL ENTERIC DYSFUNCTION BIOMARKERS AS A CLINICAL PROXY OF FUTURE GROWTH FALTERING

Najeeha Talat Iqbal[1], Kamran Sadiq[1], Sana Syed[2], Zehra Jamil[1], Kumail Ahmed[1], Junaid Iqbal[1], Aneeta Hotwani[1], Najeeb Rahman[1], Sheraz Ahmed[1], Fayaz Umrani[1], Jennie Ma[2], Indika Mallawaarachchi[2], Haberman Ziv Yael[3], Denson Lee[3], Sean Moore[2], Syed Asad Ali[1]

[1]*Aga Khan University, Karachi, Pakistan,* [2]*University of Virginia, Charlottesville, VA, United States,* [3]*Cincinnati Children's Hospital, Cincinnati, OH, United States*

Environmental Enteric Dysfunction (EED) is an acquired syndrome, an entity that encompasses environmental insults and repeated infections resulting in structural and functional abnormalities of gut. Altered gut function and poor absorptive capacity translates into linear growth faltering in children. Confirmation of EED cases requires high index of clinical suspicion, rigorous workup of malnutrition including examination of GI mucosa. In a two year longitudinal follow up study, we measured biomarkers of EED in Pakistani malnourished children. Of 350 malnourished cases, we identified 63 children who fulfilled the clinical criteria for endoscopic evaluation of upper GI mucosa with consistent features of chronic malnutrition and failure of nutritional intervention as indicated by no improvement in growth. We analyzed biomarkers of systemic and gut inflammation in fecal, urine and blood samples of EED cases who underwent endoscopy post nutrition intervention (n=52), non-EED cases (n=302) and healthy controls (n=51). In a case control comparison at 9 month of age, we observed significant differences in the median level of IGF-1(19.4 vs. 27.35; p=0.001), leptin (164.39 vs. 279.85; p<0.001), glucagon Like Peptide-2 (1133.6 vs. 1705.9; p=0.001), claudin-15 (1.56 vs. 0.91; p<0.001), ferritin (18.40 vs. 9.95; p=0.01) & pre-albumin (14.0 vs. 15.30; p=0.02) (Wilcoxon test). No differences in any biomarkers levels were observed between EED and non EED undernourished cases. Penalized linear regression and random forests approach were used to identify important variables involved in the prediction of HAZ at 24 months using a training and validation sets split in to 2/3:1/3 showing similar results in both sets. Most important predictors strongly associated with future linear growth faltering at 24 months were HAZ at enrollment (1-3 month), IGF-1, AGP, claudin-15, leptin and C-Reactive Protein. Despite being higher in malnourished cases, no gut inflammatory biomarkers measured in fecal samples predicted future linear growth faltering.

1309

EXPANDED PROGRAM ON IMMUNIZATION COMPLETION IN CHILDREN UNDER 5 YEARS AT IOM TANZANIA

Isaac Gikandi Mungai[1], Stella Mwakapotela[2], Thomas Kalasani[2], Farah Amin[1], Warren Dalal[1], Bernard Opare[2], Naoum Marwan[1]

[1]*International Organization for Migration, Nairobi, Kenya,* [2]*International Organization for Migration, Kigoma, United Republic of Tanzania*

The Expanded Programme on Immunization (EPI) was established by the World Health Organization (WHO) in 1974 to develop and expand immunization programmes throughout the world. In 1975 the government of Tanzania adopted the WHO EPI program. As part of the health assessment process for refugees in the resettlement process, the International Organization for Migration (IOM) reviews, validates and records vaccines administered to children as part of the government EPI therefore reducing over vaccination of children and allowing receiving countries to complete the catch-up schedule of their vaccine programs at a reduced cost.A review of vaccine history recorded on the US Department of State vaccination documentation worksheet was done for all refugees aged less than 5 years at the time of health assessment between October 2016 and September 2019. A child that has received measles vaccination at 9 months was defined as fully immunized.2652 children aged less than 5 years were examined before onward resettlement to the US in the study period. Of these 2485 (93.7%) had EPI vaccination records. 1864 (75.0%) were fully immunized while 167 (6.7%) were below 9 months and therefore ineligible for measles vaccination. 518 (20.8%) were not immunized. The WHO and UNICEF estimates of national immunization coverage for measles containing vaccines were90% for 2016, 99% for 2017 and 2018. Thus, the coverage rates for this group of refugees appear to be way below the national average.

1310

PREDICTORS OF DISEASE SEVERITY IN FEBRILE CHILDREN: A SYSTEMATIC REVIEW OF PROGNOSTIC STUDIES

Arjun Chandna[1], Rainer Tan[2], Michael Carter[3], Ann Van den Bruel[4], Jan Verbakel[4], Constantinos Koshiaris[5], Nahya Salim[6], Yoel Lubell[7], Paul Turner[1], Kristina Keitel[8]

[1]*University of Oxford, Siem Reap, Cambodia,* [2]*University of Lausanne, Lausanne, Switzerland,* [3]*Kings College London, London, United Kingdom,* [4]*University of Leuven, Leuven, Belgium,* [5]*University of Oxford, Oxford, United Kingdom,* [6]*Muhimbili University Health and Allied Sciences, Dar es Salaam, United Republic of Tanzania,* [7]*University of Oxford, Bangkok, Thailand,* [8]*Swiss Tropical and Public Health Institute, Basel, Switzerland*

Identifying children at risk of severe febrile illness can be challenging. Early recognition can guide referral and admission decisions, and better resource prioritization. We aimed to identify prognostic clinical features and laboratory tests that predict progression to severe disease in febrile

children presenting from the community. We systematically reviewed English language publications retrieved from MEDLINE, Web of Science and Embase between 31 May 1999 and 31 May 2019. Additional studies were identified through reference lists and consultation with domain experts. Eligibility was determined using the modified PICOTS framework. All studies evaluating clinical or laboratory prognostic factors, or clinical prediction models, in children presenting with acute febrile illness were eligible. The primary outcome was any prospectively defined objective measure of disease severity ascertained during hospitalization or within 30 days of enrollment. Risk of bias and applicability were assessed using the QUIPS and PROBAST tools. We calculated unadjusted likelihood ratios for comparison of prognostic factors and compared clinical prediction models using the area under the receiver operating characteristic curves. This study is registered with PROSPERO (CRD42019140542). Of 5,437 articles identified, 15 studies evaluating 180 prognostic factors and 14 clinical prediction models were included. Bedside coma scales (positive likelihood ratio range 1.24-14.02) and hypoglycemia (5.10-13.36) were the most common predictors of severe outcomes. Performance varied substantially across settings with different prevalence. Discrimination of clinical prediction models ranged widely (area under the receiver operating characteristic curve range 0.49-0.97). High levels of concern regarding applicability were identified and most studies were at high risk of bias. Few studies address this important public health question. Multi-site, prospective studies that include outpatients are required to identify generalizable predictors that can inform data-driven approaches to patient prioritization and triage.

1311

JOINT WEST AFRICA GROUP (JWARG) RV 466: SURVEILLANCE, DETECTION, RISKS AND CONSEQUENCES IN WEST AFRICA

Zoe Jenneh Parwon[1], Melanie McCauley[2], Abdulwasiu Bolaji Tiamiyu[3], Mihret Amare[2], Edward Nyarko[4], Suzanne Mate[5], Nkechinyere Harrison[6], Zahra Parker[7], Edward Akinwale[3], Fatoma Bolay[8], William Asiedu[4], Patricia Adams[4], Yongor Klark[9], Kara Lombardi[10], Leigh Anne Eller[10], Jayda Jones[10], Casey K. Storme[10], Jeffrey Koehler[11], Keersten Ricks[12], Randal Schoepp[12], Amy Castellano[10], Ayesha Rashid[10], Daniel Choi[10], Mark Milazzo[10], Heather Liu[10], Jarrett Headley[2], Andrew Letizia[13], Joseph Diclaro[14], Paul T. Scott[5], Nelson Michael[15], Julie A. Ake[16], Michael Iroezindu[3], Kayvon Modjarrad[5]

[1]*Camp Edward Binyah Kesselly (EBK), Margibi, Liberia,* [2]*Emerging Infectious Diseases Branch, Walter Reed Army Institute of Research; Henry M. Jackson Foundation for the Advancement of Military Medicine, Silver Spring, MD, United States,* [3]*Emerging Infectious Diseases Branch, Walter Reed Army Institute of Research, Henry M. Jackson Foundation Medical Research International Ltd/Gte, Abuja, Nigeria,* [4]*37 Military Hospital, Ghana Armed Forces, Accra, Ghana,* [5]*Emerging Infectious Diseases Branch, Center for Infectious Diseases Research, Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [6]*68 Nigerian Army Reference Hospital, Lagos, Nigeria,* [7]*Emerging Infectious Diseases Branch, U.S. Military HIV Research Program, Walter Reed Army Institute of Research; Henry M. Jackson Foundation for the Advancement of Military Medicine, Lagos, Nigeria,* [8]*National Public Health Institute of Liberia, Monrovia, Liberia,* [9]*Africabio Enterprises, Monrovia, Liberia,* [10]*Emerging Infectious Diseases Branch, U.S. Military HIV Research Program, Walter Reed Army Institute of Research; Henry M. Jackson Foundation for the Advancement of Military Medicine, Silver Spring, MD, United States,* [11]*US Army Medical Research Institute of Infectious Diseases US Army Medical Research Institute of Infectious Diseases, Fredrick, MD, United States,* [12]*US Army Medical Research Institute of Infectious Diseases, Fredrick, MD, United States,* [13]*Navy Medical Research Center, Silver Spring, MD, United States,* [14]*Navy Entomological Center of Excellence, Jacksonville, FL, United States,* [15]*Center for Infectious Diseases Research, Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [16]*U.S. Military HIV Research Program, Center for Infectious Diseases Research, Walter Reed Army Institute of Research, Silver Spring, MD, United States*

The Joint West African Research Group (JWARG) is a global partnership between military and civilian entities to detect and prevent emerging diseases of public health threats through addressing gaps in 1) research and 2) the capability to conduct research in low-resource settings. JWARG's Research Protocol RV466 has been enrolling consenting adult volunteers in Liberia, Ghana and Nigeria since 2017. Laboratory, clinical, and demographic data is collected which aids in a comprehensive understanding of the dynamics of the diseases/pathogens identified and their link to specific exposures. A total of 635 persons have been enrolled as of March 2020. There were 473 (74.49%) participants from Nigeria, 139 (21.89%) from Ghana, and 23 (3.6%) from Liberia. Of those enrolled, 339 (53.4%) were female and 296 (46.6%) were male with a median age of 35 (no difference between genders). Over half lived in urban centers (392, 62%) compared to rural areas or people living in "mixed" conditions. We looked at occupation as a function of disease risk and found that military or soldering, determined a medium exposure risk job, accounted for the highest percentage of occupations reported (17.5%). Healthcare workers (5.5%) and farmers (4.4%) accounted for some of the highest exposure risk jobs captured. Other potential exposures to disease include: non health workers with ill contacts (9.3%), recent funeral attendance (7.9%), travel (23.2%), and animal exposures within 21 days (73.8%). The majority of animal exposure was attributed to mosquito bites (69.2%). Outside of malaria, (parasites were identified in 17% of participants), there was a relative dearth of Ag, IgM and PCR for pathogen detection for viral and bacterial etiologies. However, there were high rates of IgG detection for multiple endemic and outbreak-prone diseases, (data reported elsewhere). The threat of emerging pathogens is perpetual, for appropriate bio-preparedness and response, understanding participants' exposure risk is critical.

1312

VALIDATION OF A QUALITATIVE THICK BLOOD SMEAR ASSAY AS AN ENDPOINT FOR MALARIA CLINICAL TRIALS

Peter F. Billingsley[1], Natasha KC[1], Awalludin Sutamihardja[2], Elizabeth Nyakarungu[3], Maximillian Mpina[3], Martha Sedegah[4], Arnel Belmonte[4], Tooba Murshedkar[1], Thomas L. Richie[1], Stephen L. Hoffman[1]

[1]*Sanaria Inc, Rockville, MD, United States,* [2]*The Secretariat of CCM Indonesia, The Global Fund, Indonesia,* [3]*Ifakara Health Institute and Bioko Island Malaria Elimination Program, Malabo, Equatorial Guinea,* [4]*Naval Medical Research Center, Silver Spring, MD, United States*

A *Plasmodium falciparum* (Pf) vaccine that can be deployed as part of a Pf elimination toolkit will optimally prevent Pf infection and thereby transmission to mosquitoes. Sanaria® PfSPZ Vaccine and PfSPZ-CVac, composed of aseptic, purified, cryopreserved Pf sporozoites (SPZ), have been protective against Pf infections in clinical trials in the US, Germany, and Africa. In Phase 3 clinical trials of PfSPZ Vaccine in the US, Germany and Equatorial Guinea, we will use the presence of malaria parasites in the blood, assessed by thick blood smear (TBS), as the primary clinical outcome variable to determine vaccine efficacy. We have therefore refined the preparation and reading of a TBS assay to the point of validation and transfer to field teams. For each smear, 10 µL blood are pipetted onto a 1 cm x 2 cm rectangle on a microscope slide, the blood dried then stained with 4% Giemsa, with all reagents, conditions and timings carefully controlled. The presence of parasites is assessed by reading 1 cm passes to include ≥0.5 µL blood. The TBS assay was validated using multiple operators for slide preparation and reading at multiple sites, with diagnosis being the presence of one or more parasites identified independently by two microscopists. Three expert microscopists achieved sensitivity, specificity, accuracy and precision scores of 95% to 100% when reading negative TBSs and positive TBSs at densities of 14.1-65.9 parasites/µL. Less experienced operators were trained and tested with the same slide set, and either achieved similar scores or could not be selected

as fully competent for microscopy in PfSPZ Vaccine trials. Our goal is a standardized TBS, fully validated as a cost effective assay for assessing parasitemia as an endpoint in PfSPZ Vaccine trials.

1313

MENTORSHIP TO STRENGTHEN QUALITY OF MALARIA CASE MANAGEMENT AND MALARIA IN PREGNANCY (MIP) IN ZIMBABWE: LESSONS LEARNED FROM ONE YEAR OF IMPLEMENTATION

Gilson Mandigo[1], Anthony Chisada[1], Noe Rakotondrajaona[2], Paul Matsvimbo[3], Christie Billingsley[4], Chantelle Allen[5], Katherine Wolf[5], Patience Dhliwayo[3]

[1]*ZAPIMI/Jhpiego, Harare, Zimbabwe,* [2]*ZAPIMI/Abt, Harare, Zimbabwe,* [3]*MOHCC, Harare, Zimbabwe,* [4]*PMI, Harare, Zimbabwe,* [5]*Jhpiego HQ, Baltimore, MD, United States*

Despite significant investment in training and supervision of facility-based health workers in Zimbabwe, persistent malaria case management and MIP gaps remain. National Malaria Control Program and US President's Malaria Initiative developed and implemented a mentorship intervention in five high burden malaria districts to motivate provider performance and improve quality services. From June 2018 - June 2019, 25 health workers proficient in malaria service delivery were selected and trained in clinical mentorship. These individuals mentored 98 providers at 25 facilities, covering clinical case reviews, bedside coaching, simulations, and records review. USAID's Zimbabwe Assistance Project in Malaria subsequently assessed the mentorship program through review of patient records, feedback from mentors and mentees, and engagement of stakeholders. Record review compared practices before and after implementation, using a checklist that noted completeness and appropriateness of case management across multiple parameters, including physical examination, diagnosis, classification and treatment. Mentored facilities documented improvements in recommended practices across registers: 58% to 63% for outpatient clinical settings, 53% to 64% for integrated management of neonatal and childhood illnesses, and 72% to 76% for antenatal care. A phone-based e-survey of 49 mentees and 21 mentors elicited positive feedback on the mentorship approach: 62% of mentors were "very satisfied" with the program, 67% reported quality improvement and 86% benefited from learning new skills. Among mentees: 60% were "very satisfied", 67% said that the program has improved service quality and 97% benefited from learning new skills. Common challenges included mentor transportation, mentee availability, and commodity availability. Through a review meeting, stakeholders recommended the intervention continue, as it was acceptable, feasible and achieved promising results. Recommendations include prioritizing high-volume facilities, integrating management of mentorship into District Health Executive functions and use of low-cost communication platforms to aid virtual mentorship.

1314

EVALUATION OF RATIONAL PRESCRIPTION OF ANTIBIOTICS IN A PRIMARY CARE HOSPITAL OF KINSHASA

David Pandi, Trésor Bodjick, Aline Engo, Nsengi Ntamabyaliro, Gauthier Mesia, Gaston Tona

Clinical pharmacology and pharmacovigilance unit, University of kinshasa, Kinshasa, Democratic Republic of the Congo

Antimicrobial resistance is a real concern that requires action at several levels of antimicrobial use. Overuse of antibiotics can lead to antibacterial resistance. Inappropriate indications and irrational combinations of antibiotics could contribute to the overuse of antibiotics. We conducted a cross-sectional, drug utilization study in a primary care hospital of Kinshasa in April 2019. We randomly selected 100 records. Indication for an antibiotic was considered appropriate when it was documented or based on the an antibiogram with an adequate pharmacokinetics. Irrational combinations was defined as one of the following situations: (1) antibiotics known to be antagonistic, (2) one of the antibiotics doesn't reach an active concentration at the site of infection, or (3) a combination of antibiotics that doesn't meet any of the 3 known criteria for combination. 159 antibiotics were prescribed and there were 53 cases of antibiotic combinations. 52 (32.7%, n=159) cases with inappropriate indications were identified, including 36 cases (69.2%, n=52) of antibiotic prescription in the absence of bacterial infection, 13 cases (25%, n=52) of antibiotic prescription not covering any probable germ, and 3 cases (6%, n=52) of ineffective antibiotics prescription. No antibiogram was performed. 32 (60%, n=53) cases of combinations were considered irrational, including 20 (62.5%, n=32) cases of 2 antibiotics containing 1 non-essential antibiotic, 6 cases (18. 7%, n=32) of antibiotic combinations without any bacterial infection, 5 cases (15.6%, n=32) of 3 antibiotics containing 1 superfluous antibiotic and 1 case (3.1%, n=32) of 3 antibiotics including 2 superfluous antibiotics. This study demonstrates the existence in this primary care hospital of inappropriate indications and irrational combinations of antibiotics. Given that the prescription of antibiotics is rarely based on antibiogram, both because of therapeutic emergencies and weak diagnostic tools in developing countries, probabilistic antibiotic therapy remains predominant. Thus, it is necessary to strengthen the prescribers in the rational prescription of antibiotics.

1315

UNDERLYING CAUSES OF PERINATAL DEATHS AMONG CASES UNDERGOING MINIMALLY INVASIVE TISSUE SAMPLING IN BANGLADESH

Afruna Rahman[1], Kyu Han Lee[2], Sanwarul Bari[1], Farzana Islam[1], Mustafizur Rahman[1], Muntasir Alam[1], Tonima Islam Trisa[1], Shams El Arifeen[1], Emily S. Gurley[2]

[1]*icddr,b, Dhaka, Bangladesh,* [2]*John Hopkins Bloomberg School of Public Health, Baltimore, MD, United States*

About 40% of global infant mortality is attributable to perinatal deaths, including stillbirths and deaths within the first six days of life. In 2014, the perinatal mortality rate was 44 per 1000 pregnancies in Bangladesh. Underlying factors remain less explored due to lack of standardized vital registration system. Child Health and Mortality Prevention Surveillance (CHAMPS) is a multi-country programme that aims to understand and track the preventable causes of childhood deaths in seven countries, through postmortem Minimally Invasive Tissue Sampling (MITS) and other information. The objective of our analysis was to explore underlying causes of perinatal deaths among cases undergoing MITS in Bangladesh. From October 2017 to February 2020, an expert panel determined underlying cause for individual MITS cases based on histopathology, molecular and microbiological diagnostics, clinical data abstraction, and verbal autopsies. We present data for 117 perinatal deaths; all except two occurred at three health facilities. Fifty-seven were stillbirths (49%) and 60 were early neonatal deaths (within 6 days of birth). Intrauterine hypoxia was the major underlying cause (46/57) of death for the stillbirths, with 22% (10/46) of these due to placental complications. Other stillbirth causes included maternal hypertension, intrauterine infection, and labor and delivery-related complications. Out of three stillbirths due to intrauterine infection, two were caused by Streptococci, one by Varicella Zoster. Half of early neonatal deaths were due to preterm birth complications and 33.3% (20/60) were due to perinatal asphyxia. Six neonatal deaths resulted from neonatal sepsis caused by *Klebsiella pneumoniae*. Of all 117 perinatal deaths, 92.35% (108/117) were considered preventable. Expert panel considered 60 of 108 deaths as preventable through regular quality antenatal care (ANC), as only 22 cases had a history of any ANC visit, 21 cases had no history and for others, ANC status was unavailable. Suboptimal ANC access in the community highlights the need to improve these services in rural Bangladesh to reach targets for reducing child mortality.

1316

NEUROCOGNITIVE IMPAIRMENT IN UGANDAN CHILDREN UNDER 5 YEARS OF AGE WITH CEREBRAL MALARIA OR SEVERE MALARIAL ANEMIA OCCURS EARLY AND PERSISTS TWO YEARS AFTER ILLNESS

Paul Bangirana[1], Dibyadyuti Datta[2], Andrea L. Conroy[2], Yi Zhao[2], Ruth Namazzi[1], Robert O. Opoka[1], Chandy C. John[2]

[1]*Makerere University, Kampala, Uganda,* [2]*Indiana University, Indianapolis, IN, United States*

Severe malarial anemia (SMA) and cerebral malaria (CM) have been associated with neurocognitive impairment in children in sub-Saharan Africa, but the length of this impairment in children <5 years is not well defined. The present study assessed neurocognitive function in children with CM or SMA 1 week after discharge, and then at 6-, 12- and 24-months follow-up. Children below 5 years with SMA (n=196) or cerebral malaria (CM) (n=204) were recruited from Mulago Hospital in Kampala, Uganda from November 2008 to December 2013 and followed for 24 months. Healthy community control children (CC, n=172) were also recruited from homes and neighbourhoods of the children with malaria. Children were assessed using the Mullen Scales of Early Learning for overall cognitive ability, the Early Childhood Vigilance Test for attention and the Colour Object Association Test for memory. Test scores were converted into age-adjusted z-scores based on scores of the CC. Linear mixed effects regression models were used to compare test scores between the groups. At 24-month follow-up, children with SMA had poorer scores than the CC for overall cognitive ability (estimated mean difference, -1.33, 95% CI: -1.78 to -0.88, p <0.0001) but not for attention and memory. Children with CM had poorer scores for cognitive ability (-1.17, 95% CI: -1.65 to -0.69, p<0.0001) and memory (-0.80, 95% CI: -1.14 to -0.45, p<0.0001) but not for attention than CC. For cognitive ability in SMA or CM, and memory in CM, z-scores were also worse at baseline, 6- and 12-month time points compared to CC. These findings suggest that neurocognitive impairment in overall cognitive ability occurs early in CM and SMA and persists for at least 2 years after the episode. These findings call for further studies to develop adjunct therapies to prevent neurocognitive impairment during and after the disease episode in children with CM or SMA, and to provide interventions for those identified as at risk for long-term impairment.

1317

GALECTIN 3 AS A PREDICTOR OF CLINICAL AND DEATH OUTCOMES AMONG CHAGAS DISEASE PATIENTS IN A PROSPECTIVE COHORT

Carlos H. Moreira[1], Lea C. De Oliveira[1], Ana L. Bierrenbach[1], Clareci Cardoso[2], Claudia Di Lorenzo[2], Ariela M. Ferreira[3], Maria C. Nunes[4], Desiree S. Haikal[5], Antonio Ribeiro[4], Fabio Fernandes[6], Nayara D. Quintino[2], Ester C. Sabino[1], Thallyta M. Vieira[1]

[1]*Institute of Tropical Medicine - University of Sao Paulo, São Paulo, Brazil,* [2]*Federal University of Sao Joao Del Rei, Minas Gerais, Brazil,* [3]*Universidade Estadual de Montes Claros, Minas Gerais, Brazil,* [4]*Federal University of Minas Gerais, Minas Gerais, Brazil,* [5]*Universidade Estadual de Montes Claros., Minas Gerais, Brazil,* [6]*Heart Institute - inCOR - University of Sao Paulo, São Paulo, Brazil*

Chagas heart disease (CHD), the leading cause of infectious myocarditis worldwide, plays a significant yearly mortality burden ~ 12,000 death. CHD shows great remodeling, fibrosis, hypertrophy, and tissue damage. Galectin-3 (GAL-3), by proxy, can translate fibrogenesis potential under certain stimuli. It's levels act as a biomarker to stratify who could benefit from early approaches. We aim to evaluate if GAL-3 levels are associated with the development of ECG abnormalities, death, and NT-proBNP changing. We analyzed data from the SaMi-Trop cohort study in a highly endemic Brazilian region. Methods: We collected baseline sociodemographic and clinical data, blood samples for the measure of GAL-3 and ECG analysis. The cohort was followed-up for a median of 24 months, and we reaccessed baseline data, as well as mortality. We ran Cox regression adjusted for age and sex to check the HR for all-cause death, including GAL-3 strata, in composite to abnormal NT-proBNP by age, and CD suggestive ECG findings. Results: From 1813 patients, we excluded 493 previously treated with benznidazole, resulting in 1320 patients included: 782 (59.6%), low risk strata (GAL-3 ≤ 17.8 ng/mL); 423 (32.2%), moderate risk (17.8-25.9 ng/mL), and 108 (8.2%) high risk (> 25.9 ng/mL). GAL-3 median and IQR levels of 18.2 [14.3-23.1] among the 100 (7.6%) deceased, while 16.5 [13.6-20.1] ng/mL among survivals (p<0.001). In addition, median/IQR NT-proBNP baseline were higher among High risk strata 390,5 [109-1407] pg/mL (p<0.001). CHD suggestive ECG findings were more frequent in the high-risk strata 79(73%) p<0.001. For higher-risk strata, HR 2.1(95% CI,1.2-3.7). The composite of ECG suggestive of CHD and GAL-3 high levels resulted in HR 12 (95% CI,4.2-39). Likewise, ECG suggestive and normal NT-proBNP adjusted by age and High GAL-3 levels, resulted in HR 6.4 (95% CI, 1,8-23), while high NT-proBNP adjusted by age and higher Gal-3 strata HR 27 (95% CI, 8.4-87.4). Data suggest that GAL-3 could be integrated into the management of CHD as an earlier biomarker to assess the risk of death and to identify higher-risk ones with ECG abnormalities in whom NT-proBNP is not already abnormal.

1318

PREVALENCE OF ILLNESSES IN HEALTHY ADULTS IN BANCOUMANA, MALI

Jessica Y. Aduwo[1], Jennifer L. Kwan[2], David M. Cook[1], Rathy Mohan[1], Sara Healy[1], Mahamadoun H. Maiga[3], Mamady Kone[3], Kourane Sissoko[3], Issaka Sagara[3], Ogobara K. Doumbo[3], Patrick E. Duffy[1]

[1]*Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States,* [2]*Epidemiology Unit, Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States,* [3]*Malaria Research and Training Center, Mali-NIAID ICER, University of Science, Techniques and Technologies of Bamako, Bamako, Mali*

The burden of communicable diseases in Africa has decreased in recent years but remains high. We performed a secondary analysis on data from malaria vaccine trials conducted in Bancoumana, Mali to quantify the prevalence of diseases in healthy African adults. These trials (NCT01867463 and NCT02334462) were conducted from May 2013-March 2015 and April 2015-September 2017, respectively and evaluated malaria transmission-blocking vaccine candidates, Pfs25M-EPA and Pfs230D1-EPA. Bancoumana is a rural town of about 9000 people, with intense malaria transmission during the rainy season from June to October. Participants with evidence of chronic disease such as hypertension, HIV, HBV, or HCV infections, abnormal ECGs, or abnormal hematologic, renal or hepatic laboratory indices were excluded from the trials. After enrollment, adverse events (AEs), including intercurrent illnesses, were coded according to the standard Medical Dictionary for Regulatory Activities (MedDRA). The analytical population included 60 and 61 participants enrolled in the comparator arms (receiving TWINRIX, Menactra, Euvax B and/or normal saline), respectively. Only AEs that were determined to be unrelated to vaccine administration were considered for this initial analysis. Monthly rates were calculated for the 10 most frequent illnesses, which were malaria, cold, headache, rhinitis, gastritis, tooth decay, rhinobronchitis, conjunctivitis, bronchitis, and back pain, indicating the high frequency of infections in a healthy adult population. Among the three most common infections, the proportion of individuals with at least one episode of malaria was 76.7% and 73.8% in the two trials respectively; with cold was 60% and 54.1%; and with rhinitis was 83.3% and 31.1%. Both trials exhibited similar proportions (assessed by chi-square), except rhinitis (p=0.027). While malaria displayed a clear seasonal pattern, the other illnesses did not. These analyses are informative for understanding the prevalence of commonly reported illnesses experienced by adults in Bancoumana and may help inform future preventive actions.

1319

POTENTIAL IMPACT OF COVID-19 SUPERININFECTIONS

Looli Hassan Alawam[1], Aliou Bouba[2], Girma M. Zelleke[3], Ibrahim B. Toheeb[1], Vincent Appiah[4], Arlinda Sadiku[5], Kristina B. Helle[6], Gideon Ngwa[7], Martin Eichnner[1], Kristan Schneider[6]

[1]*University of Tubingen, Tubingen, Germany,* [2]*University of Mittweida, Mittweida, Germany,* [3]*African institute for mathematical science, Limbe, Cameroon,* [4]*West African Centre for Cell Biology of Infectious Pathogens, University of Ghana, Ghana, Ghana,* [5]*Mittwieda unversity, Mittweida, Germany,* [6]*Mittweida university, Mittweida, Germany,* [7]*University of Bouia, Bouia, Cameroon*

In response to the global COVID-19 pandemic governments had to implement controversial control mechanisms. The path of aiming for herd immunity by letting the disease spread and only protecting vulnerable groups, which is particularly debatable, has been followed by some policy makers, most prominently in the UK. While confronting the pandemic peak, leads to a high number of recovered and hence immunized individuals, this argument harbors intrinsic flaws. With the viral population growing, genetic diversity increases, and so do multiple infective contacts. The effects of super-infections with slightly different pathogen variants on the course of the disease are yet not understood. Namely, such infections can potentially lead to an elevated risk of episodes being severe or lethal. As observations are limited, models can help to predict the impact of such factors' potential risks. We extend the compartmental model from the pandemic preparedness tool CovidSim (http://covidsim.eu/) model by accounting for multiple infections, being associated with an elevated risk of developing severe and lethal episodes. The model helps to predict the demand of hospital and ICU capacities and allows to make inferences about the economic damage caused by a pandemic peak. We present simulation results obtained with the software Berkely Madonna concerning the impact of different control interventions on the height and delay of the pandemic peak. These results further allow to deduce economic implications of the implemented interventions.

1320

SYNERGY BETWEEN MALNUTRITION AND INFECTION PROMOTES BONE RESORPTION IN MICE

E. Yaneth Osorio[1], Zbigniew Gugala[1], Genesis Palacios[2], Erika Cordova[1], Ashanti Uscanga-Palomeque[1], Grace E. Patterson[1], Bruno L. Travi[1], Peter C. Melby[1]

[1]*University of Texas Medical Branch, Galveston, TX, United States,* [2]*Universidad de La Laguna, Tenerife, Spain*

The synergy between malnutrition and infection is a major contributor to childhood morbidity and mortality. We used a murine model of moderate acute malnutrition (MAM) to investigate the effects of MAM and acute infection or inflammation on bone. Malnourished mice (MN) challenged with *Leishmania donovani* or bacterial lipopolysaccharide (LPS) were compared to naïve MN mice and/or well-nourished (WN) control mice at 72 and 24 hrs post-challenge, respectively. We found that MN-mice had reduced femur and tibia length compared to WN mice ($p<0.001$). A positive correlation of femur length to body length observed in WN mice was absent in MN mice. Bone marrow (BM) CD3+RANKL+ cells, which are critical for differentiation of bone-resorbing osteoclasts, were increased after challenge of MN mice compared to both naïve MN mice and challenged WN mice ($p<0.05$). Tartrate-resistant acid phosphatase activity (TRAP+) in was higher in the sternums of challenged MN mice compared to naïve MN-mice ($p<0.01$). This indicates increased osteoclast activity and bone resorption when the MN host is infected. Increased levels of inflammatory markers (*crp*, *il1b*, *il18*, and *Tnfa*), and a higher frequency of inflammatory monocytes were found in BM of challenged MN mice compared to challenged WN mice. This suggests that inflammation in the MN host drives bone resorption. MN and WN mice had similar proportions of BM mesenchymal stem cells (MSC; CD45-CD29+), which differentiate into either adipocytes or osteoblasts. However, the frequency of Oil red O positive adipocytes and the expression of genes associated with adipocyte differentiation, *(Adypoq* and *Srbp1),* was increased in the BM after challenge of MN mice. These data suggest bone formation is hindered because BM MSCs differentiate to adipocytes rather than to osteoblasts. We conclude that inflammation leads to enhanced osteoclast activity and preferential differentiation of MSCs to fat cells over osteoblasts, which results in reduced bone formation and increased resorption in the MN host exposed to acute infection.

1321

EFFECT OF ANTENATAL PARASITIC INFECTIONS ON ANTI-POLIO IGG LEVELS IN CHILDREN: A PROSPECTIVE BIRTH COHORT STUDY IN KENYA

Indu Malhotra[1], A. Desiree LaBeaud[2], Peter L. Mungai[1], Maxim J. McKibben[3], Xuelei Wang[1], Nathan Morris[4], Eric Muchiri[5], Christopher L. King[1], Charles H. King[1]

[1]*Case Western Reserve University, Cleveland, OH, United States,* [2]*Stanford University, School of Medicine, Stanford, CA, United States,* [3]*North Carolina Neurosciences Hospital, Chapel Hill, NC, United States,* [4]*Eli Lilly and Company, Indianapolis, IN, United States,* [5]*Meru University of Science and Technology, Nairobi, Kenya*

Vaccination studies have repeatedly shown that children in developing nations are less responsive to vaccines than children from developed countries. This disparity is especially apparent with respect to oral vaccines such as oral polio vaccine. One potential contributing factor is the presence of maternal antenatal infections, which can modulate the infant's developing immune system. We examined the effect of malaria and helminth infection in pregnant women on the development of antibody responses to polio in their offspring following vaccination. Children enrolled in a birth cohort in rural coastal Kenya received four trivalent polio vaccinations before 6 months of life. 232 Kenyan women were tested for malaria, lymphatic filariasis (LF), urogenital schistosomiasis, hookworm and helminths during pregnancy. Plasma samples were collected from their 232 offspring every 6 months from birth to 3 years of age and tested for levels of IgG against polio at each time point. Overall, one third of the mothers were infected with LF, urogenital schistosomiasis, malaria or hookworm. Overall 58% of the pregnant women were infected with helminths; LF (18%) was the most common class of parasitic infection, followed by malaria (13.4%), hookworm (11.2%), schistosomiasis (10.8%), *Trichuris* (4%), prevalence of *Strongyloides* and *Ascaris* was <1%. Offspring of malaria-infected women had higher anti-polio IgG at 6 and 30 months of age ($P=0.02$ and 0.03, respectively) compared to offspring born to women without malaria. There was no significant difference in anti-polio IgG in children of mothers infected with schistosomiasis or LF as compared to children of uninfected mothers. Antenatal maternal helminth infections were not associated with reduced antibody responses to infant polio, but rather with modestly increased IgG responses to oral polio vaccine. Further analysis of the data is in progress.

1322

THE EFFECTS OF AGE, SEX, AND INDIVIDUALITY ON THE BLOOD TRANSCRIPTOME IN NICARAGUAN CHILDREN: DOES BASELINE PREDICT INFECTION OUTCOME?

Stephen J. Popper[1], Nicholas Rachmaninoff[2], Neha Bansal[2], Lionel Gresh[3], Galina Koroleva[2], Aubree Gordon[4], Guillermina Kuan[5], Angel Balmaseda[6], John Tsang[2], Eva Harris[1]

[1]*Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, Berkeley, CA, United States,* [2]*Center for Human Immunology and Multiscale Systems Biology Section, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States,* [3]*Sustainable Sciences Institute, San Francisco, CA, United States,* [4]*Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, Ann Arbor, MI, United*

States, [5]Health Center Sócrates Flores Vivas, Ministry of Health, Managua, Nicaragua, [6]National Virology Laboratory, National Center for Diagnosis and Reference, Ministry of Health, Managua, Nicaragua

The state of the immune system varies widely across individuals and populations, and contributes to differences in the response to infection, vaccination, and other stimuli. Recent studies have begun to highlight how age, sex, genes, and environment shape the immune system, but their contribution to immune variation is still poorly understood, particularly in children and in low- and middle-income countries. Here we generated a large-scale longitudinal blood transcriptome dataset from a long-standing pediatric cohort study in Nicaragua. We obtained RNA-Seq data on 679 blood samples from 115 healthy children sampled each March for six consecutive years in 3 overlapping age cohorts (ages 1-5 or 2-6, 5-10 and 9-14), and dissected the source and structure of gene expression variation. We observed clear age-dependent trends, though for most genes this was dominated by subject-specific patterns of expression, even after controlling for sex. The expression of B cell-associated genes decreased with age, particularly in the 1-5 age group, and expression of neutrophil genes increased with age, while NK cell- and monocyte-associated genes had strong subject-specific patterns of expression and were also associated with differences in growth status. Extensive information exists on the history of viral infections and other health outcomes in the Nicaraguan cohort. To examine the potential for linking baseline gene expression with infection outcomes, we compared gene expression in children who then had a symptomatic (n=26) or clinically inapparent (n=12) infection during successive chikungunya epidemics that occurred in 2014 and 2015. Genes that differed in expression in those who went on to have symptomatic vs inapparent infections were more subject-specific, suggesting that temporally stable features of the personal transcriptome may contribute to the response to infection. These findings show that individuals can possess an intrinsic gene expression profile over multiple years and illustrate the potential for using baseline gene expression features to better understand and predict health and disease outcomes.

1323

IDENTIFICATION OF GENETIC LOCI FOR BLOOD LIPID LEVELS IN INDIVIDUALS OF AFRICAN ORIGIN

Sounkou M. Toure[1], Jeffrey G. Shaffer[2], Cheickna Cisse[1], Mamadou Wele[1], Seydou O. Doumbia[1], Segun Fatumo[3], Aboubacrine M. Toure[1]

[1]*University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali,* [2]*Tulane University, New Orleans, LA, United States,* [3]*London School of Hygiene & Tropical Medicine, London, United Kingdom*

Despite the increasing public health burden of cardiovascular diseases (CVD) in sub-Saharan Africa and African ancestry populations globally, the genetic epidemiology of cardio-metabolic traits remains dramatically underdeveloped in those populations despite recent efforts. Most genome wide association studies (GWAS) of CVD have been performed on Eurasian populations, and studies involving subjects of African ancestry individuals were primarily used for replication or fine mapping. Previous studies have shown that GWAS findings may not be generalizable from Eurasian to African populations, particularly for involving genetic risks variants of major risk determinants of CVD such as serum cholesterol, triglycerides, low and high density lipoproteins levels. Several explanations have been put forth for explaining this phenomenon, specifically allele and linkage disequilibrium differences, admixture effects, natural selection, and gene-environment interplay. Further, most of the current GWAS studies are not focused on clinical related traits like serum lipids levels (SLT) in African populations. Utilizing GWAS data resources from Uganda, South Africa, Ghana, and Nigeria multivariate and gene-level genome wide association methods accounting for the relation among SLT, we identified loci that were previously undetected by the standard analytical methods. Beyond the replication of known blood lipid related genes like APOE, PCSK9, LIPC, multivariate methods were used to identify the HAVCR1 as a significant gene, which suggests the relevance of viral load in the epidemiology of blood lipids regulation in African populations.

1324

IMPACT OF SEVERE MALARIA, IRON THERAPY AND OXIDATIVE STRESS ON RISK OF READMISSION AND DEATH IN AFRICAN CHILDREN

Daniel B. Blatt[1], Ian C. Michelow[1], Katrina Co[2], Dibyadyuti Datta[2], Caitlin Bond[2], Robert O. Opoka[3], Sarah E. Cusick[4], Chandy C. John[2]

[1]*Brown University/Hasbro Children's Hospital, Providence, RI, United States,* [2]*Indiana University School of Medicine, Indianapolis, IN, United States,* [3]*Makerere University, Kampala, Uganda,* [4]*University of Minnesota, Minneapolis, MN, United States*

P. falciparum malaria is a major cause of morbidity and mortality in African children. Severe cases may be complicated by cerebral malaria (CM) and severe malaria anemia (SMA). In addition to effective antimalarial therapy, the WHO recommends routine supplementation with iron due to the high prevalence of iron deficiency (ID) in Africa. In this context, severe malaria and iron therapy are associated with disruption of the redox equilibrium due to oxidative stress. This study was designed to assess the impact of severe disease (CM or SMA) and biomarkers of oxidative stress on death during admission, and on all-cause readmissions up to 6 months post-discharge. Children <5 years of age with ID (zinc protoporphyrin >80 µg/dL) and CM (n=79) or SMA (n=77) were enrolled from Mulago Hospital in Kampala, Uganda. We randomized 156 children to receive a 3-month course of iron either starting immediately (CM, n=39; SMA, n=39) or 28 days after malaria diagnosis (CM, n=40; SMA, n=38). Serum heme oxygenase-1 (HO-1) and malondialdehyde (MDA), markers of oxidative stress, and superoxide dismutase activity (SOD), an antioxidant measure, were tested at admission and 28 days later. To identify predictors of death during initial hospitalization, we used binary logistic regression analyses for CM and for SMA using models for each surrogate of oxidative stress, adjusted for age. Among children with CM, 9 of whom died during admission, a log increase in HO-1 led to increased mortality (OR=6.1, CI=1.2-31.3), while a log increase in SOD led to decreased mortality (OR=0.02, CI=0.001-0.70). There were no deaths in children with SMA in this study. After adjusting for age and iron therapy, in children with CM or SMA, there were no associations between biomarkers of oxidative stress during initial admission or 28 days later and readmission within 6 months. In conclusion, in children with CM, HO-1, a biomarker of oxidative stress, was associated with increased odds of death and antioxidant activity (SOD) was associated with decreased odds of death. Measures of redox balance may be useful in predicting mortality in children with severe malaria.

1325

NUTRITIONAL STATUS AND VACCINE RESPONSE IN CHILDREN LIVING IN URBAN SLUMS OF MUMBAI

Samantha Lee Huey, David G. Russell, Jere D. Haas, Julia L. Finkelstein, Saurabh Mehta

Cornell University, Ithaca, NY, United States

Infectious diseases, such as measles and polio, are particularly severe in children under age 5, especially in settings such as urban slums. Higher anti-measles or polio immunoglobulin (IgG) titer lowers the likelihood of developing severe measles or polio disease, but how nutrition is associated with IgG titers is less understood. Among 12-24-month-olds with vaccine records and IgG titer data in urban slums of Mumbai who were enrolled in a randomized biofortification trial, we sought to determine the association of nutritional status (anthropometry and micronutrient biomarkers) and measles and polio IgG titers. Demographics, health history, and anthropometry were assessed and serum C-reactive protein (CRP), vitamins A, B12, D; ferritin; zinc; measles and polio IgG were. Associations between nutritional correlates and IgG titer were assessed by multivariate linear regression. At baseline (age 15 mo), 65% were protected against measles (IgG >16.5 AU/mL, n=77) while 86% had protective polio IgG (>12 U/mL, n=22). Age was positively associated with measles IgG, while vitamin D deficiency [25(OH)D <50 nmol/L] was associated with lower measles IgG. Lower polio IgG titer was associated with CRP >5 mg/L,

report of diarrhea, and maternal age. At endpoint (age 24 mo), 86% had protective measles IgG titer (n=87) and 87% had protective polio IgG (n=24). Baseline vitamin D, maternal height, and underweight predicted lower measles IgG at endpoint, while age and report of cough predicted higher measles IgG. Baseline predictors of lower polio IgG at endpoint included maternal height and underweight, while higher polio IgG was associated with the trial intervention arm. Protective measles IgG titer was lower than expected, despite vaccination at age 9 mo, which is in line with recommendations. In addition to factors such as age, illness, and maternal height, deficiency in vitamin D and underweight were associated with IgG titer, highlighting the potential role of nutrition in vaccine response.

1326

PILOT IMPLEMENTATION OF ICD - 10 BASED ELECTRONIC CLINIC REPORTING SYSTEM FOR A DEVELOPING COUNTRY AT SHALOM FAMILY MEDICINE CLINIC IN SANTIAGO TEXACUANGO, EL SALVADOR

Maria Jose Lopez[1], Teri Benner[2], Dean Sutphin[3], Harold Garner[4], Cameron Sumpter[4]

[1]*Shalom Family Medical Center, Santiago Texacuangos, El Salvador,* [2]*Shalom Family Medical Center, Harvesting in Spanish, Santiago Texacuangos, El Salvador,* [3]*Edward Via College of Osteopathic Medicine, Blacksburg, VA, United States,* [4]*Edward Via College of Osteopathic Medicine, Spartanburg, SC, United States*

Developing country primary care family medical clinics need affordable electronic reporting. In response, Via College of Osteopathic Medicine (VCOM) developed an Electronic Clinical Record (ECR) to provide an efficient, user-friendly record of physician diagnosis, procedures, and medications during patient encounters. Design features sought efficient accurate reports to manage patient care, clinic planning, epidemiological analyses, preparing for seasonal diseases, detecting disease outbreaks, communicating with key stakeholders and meeting required reporting. Shalom Family Medical Center in El Salvador needed reports for the El Salvador Ministry of Health, affiliated Medical Schools to provide teaching center activities, and reports to Clinic governing board. The aim was to pilot ECR January - April 2019 to assess viability, challenges and successes of a north-south VCOM and Shalom Clinic collaboration. A project director facilitated daily physician training, team meetings, videoconference with US experts, consultation, verification of accuracy by comparison of manual and electronic records, elimination of duplications and omissions, identification, and resolution of obstacles, troubleshooting software and repairs. Review included affordable, user-friendly, physician real-time patient entry by physician username and password protection. Information includes gender, age, location, vital signs, body mass index (BMI), type of visit, laboratory exams requested; diagnosis by ICD-10, procedures, and medications. After 3 months, physicians demonstrated independent, accurate, and efficient use of ECR. By the beginning of April 2019, utilizing data outputs on 758 patients including 495 female and 263 male patients, a report was produced for the annual board meeting. Data were provided as a baseline proof of concept for database viability for 800-1000 patients per month. Further, prototype monthly, quarterly and annual reports were developed. This prototype development of ECR provided the baseline for Shalom Center and facilitated deployment to other VCOM affiliated clinics in Honduras and Dominican Republic.

1327

PERCEIVED BARRIERS TO CHAGAS SCREENING AMONG OBGYN AND FAMILY MEDICINE PROVIDERS

Helen Mahoney West[1], Carly Milliren[1], Olivera Vragovic[2], Julia Köhler[1], Christina Yarrington[2]

[1]*Boston Childrens Hospital, Boston, MA, United States,* [2]*Boston Medical Center, Boston, MA, United States*

Chagas disease is a neglected disease of poverty transmitted by an insect vector that infests substandard housing in the continental Americas. 3,400 residents of Massachusetts are estimated to have Chagas disease which leads to cardiac death in 20-30% of those who remain untreated. Congenital transmission is thought to occur in 1 – 10% of pregnancies. Screening at-risk populations and treating individuals with Chagas disease is key to prevent complications and optimally manage heart disease once it has occurred. Identifying infection in pregnant women facilitates treatment of both mother and infant if congenital infection is detected. Following an information session on Chagas disease, Obstetrics/Gynecology staff at Boston Medical Center decided to screen their at-risk pregnant patients. To our knowledge, Boston Medical Center and its referring facility, East Boston Neighborhood Health Center, are the only medical facilities in the United States to incorporate Chagas screening and treatment into standard clinical obstetrics practice, partnering with adult and pediatric Infectious Disease staff. To identify screening barriers, an anonymous survey was distributed to 178 Obstetrics/Gynecology and Family Medicine practitioners at Boston Medical Center. The 37% (n=66) response rate was likely impacted by the onset of the COVID-19 pandemic. The majority of respondents (64%) reported being familiar with Chagas disease. However, only 32% knew how to order a test; 22% reported knowing what to do if a test was positive. Only 26% were familiar with the time needed to educate patients. The majority of respondents think Chagas screening is very (39%) or somewhat (48%) important as a public health initiative and 33% judged screening patients at the time of clinic visits as very important. These findings will be incorporated into our measures to facilitate full implementation of Chagas screening, and can inform initiatives at other centers who wish to address this deeply neglected infection among their patient families.

1328

CHALLENGES TO COVID-19 DIAGNOSTICS IN HAWAI'I

Lauren L. Ching[1], Katy D. Hintzen[2], Rosie Alegado[3], Vivek R. Nerurkar[1]

[1]*Department of Tropical Medicine, Medical Microbiology, and Pharmacology, John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, HI, United States,* [2]*University of Hawaii Sea Grant College Program, School of Ocean and Earth Sciences and Technology, University of Hawaii at Manoa, Honolulu, HI, United States,* [3]*Department of Oceanography and Center for Microbial Oceanography, School of Ocean and Earth Sciences and Technology, University of Hawaii at Manoa, Honolulu, HI, United States*

The coronavirus disease 2019 (COVID-19) pandemic has posed unique challenges to the state of Hawai'i, due to the state's geographic isolation, economic dependence on tourism, and clinical laboratory infrastructure. Further, Hawai'i is the only state surrounded by the Pacific Ocean. As of April 22, 2020, there have been 592 confirmed cases of COVID-19 and 12 deaths in the state of Hawai'i. Over 80% of the cases have been associated with travel or travel-associated contact. Thus, in addition to a statewide "stay-at-home" order, the state has implemented travel restrictions on all non-essential travel both out-of-state, as well as inter-island, requiring all travelers either out of the state or within the state to undergo at least 14-day quarantine. While essential, these restrictions have uniquely affected Hawai'i's economy due to the state's economic dependence on tourism. A state-by-state analysis of unemployment in the nation has concluded that Hawai'i has had the highest rate of unemployment in the nation, estimating that nearly 25% of the state's labor force filed for unemployment in the last month. Thus, there is a pressing need to increase testing capacity for SARS-CoV-2, the causative agent of COVID-19, so that workers can safely return to their jobs, and the tourism industry can recover. The state's clinical laboratory capacities and infrastructure have resulted severe testing shortages and extended delays in turn-around times for individuals to receive their tests results. Most diagnostic tests in the state of Hawai'i are sent to laboratories on the continental US due to lack of facility to conduct the high complexity molecular diagnostic testing required for SARS-CoV-2 detection within the commercial clinical laboratories in the state. However, expertise and infrastructure facility are available within research laboratories in the state. In this report we discuss the challenges and opportunities to build strong

collaborations between the state Department of Health, commercial clinical laboratories and research laboratories to combat the ongoing COVID-19 pandemic and future threats from emerging infectious diseases.

1329

SURVIVAL OF LIVE BORN BABIES BY GESTATIONAL AGE AND BIRTH WEIGHT FOR AGE 0-60 DAYS AMONG DELIVERIES AT A TERTIARY HOSPITAL IN ETHIOPIA

Delayehu Bekele[1], Kimi Van Wickle[2], Gesit Metaferia[1], Tefera Biteye[1], Yahya Mohammed[1], Oludare A. Odumade[3], Mahlet Abayneh[1], Grace J. Chan[2]

[1]*St. Paul's Hospital Millenium Medical College, Addis Ababa, Ethiopia,* [2]*Harvard T.H. Chan School of Public Health, Boston, MA, United States,* [3]*Boston Children's Hospital, Boston, MA, United States*

Neonatal death accounts for 40% of deaths under the age of 5 years worldwide. Estimates of survival by birth weight and gestational age in low-income countries are important for policy-makers and clinicians. The aim of this study is to determine gestational age- and birthweight-specific survival rates among babies born at Saint Paul's Hospital Medical College in Addis Ababa, Ethiopia up to 60 days of life. As part of a larger multi-country study on the Burden of Antibiotic Resistance in Neonates from Developing Societies (BARNARDS), we conducted a prospective cohort study at St. Paul's Hospital Millennium Medical College (SPHMMC) in Addis Ababa on 4,583 mother-newborn pairs for births between March 2017 and February 2018. Newborns were followed over the first 60 days of life. The primary outcome measure was time-to-death, defined as death within 60 days of life. Time-to-death was assessed using Kaplan-Meier survival analysis and the predictors of neonatal mortality were measured as adjusted hazard ratios using Cox regression models. 1.8% of neonates died within that first week of life and an additional 0.3% died within the 60 days of life. The overall median time to death was 3.0 days. After adjusting for sociodemographic, obstetric, and newborn characteristics, we found that vaginal breech(AHR 6.8[1.2;32.8]), elective c-section (AHR 5.8[1.7;19.7]), and meconium-stained amniotic fluid (by observation) (AHR 2.9[1.2;7.0]) were significantly associated with increased risk of death within 60 days of life. Survival rates through 60 days of follow-up were 33% for newborns with birthweights <1000g, 52% for newborns 1000-<1500g, 95% for newborns 1500-<2500g, and 99% for newborns 2500 - <4000g. Like in other contexts, LBW (AHR 3.4[1.2;9.6]) was found to be a strong predictor of death. However, in Ethiopia LBW is less prevalent. We found 0.20 % of newborns were <1000g, 1.36% were 1000-<1500g and 15.45% were 1500g-<2000g. Our analysis suggest that low birth weight is a strong predictor for survival in the Ethiopian context.

1330

DIETARY OMEGA-3 FATTY ACIDS AMELIORATE PATHOLOGICAL INFLAMMATION IN A MODEL OF MODERATE ACUTE MALNUTRITION

Grace E. Patterson, E. Yaneth Osorio, Alex Peniche, Sara M. Dann, **Peter C. Melby**

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

We previously established a mouse model of moderate acute malnutrition (MAM) with a diet deficient in protein, energy, zinc, and iron. Malnourished (MN) mice exhibited impaired intestinal barrier function, increased systemic inflammation, and an exaggerated inflammatory response to bacterial lipopolysaccharide (LPS). These pathophysiological features were driven by a proinflammatory intestinal microbiota (increased Proteobacteria but decreased Bacteroidetes). Treatment of acute childhood malnutrition often includes energy-dense lipid-based supplements. They are high in omega-6 polyunsaturated fatty acids (PUFAs) but contain negligible amounts of omega-3 PUFAs. We hypothesized that replacement of the omega-6-rich corn oil in the MN diet with omega-3-rich fish oil could ameliorate the exaggerated inflammation in MAM. Malnourished (MN) mice with dietary corn oil (MNCO) were compared with MN mice with dietary fish oil (MNFO) and well-nourished (WN) control mice. Following challenge with LPS, MNFO mice showed reduced expression of inflammatory cytokines and less weight loss than MNCO mice. MNFO mice showed significantly less spontaneous bacterial translocation to the liver compared to MNCO mice (p = 0.016) and displayed increased intestinal expression of the antimicrobial proteins *Reg3b* and *Reg3g,* and cytokines *Il17* and *Cxcl1*. MNFO mice also had increased intestinal *Cldn3* and reduced *Hapt* expression, indicating improved intestinal barrier function. The cecal microbiota composition of MNFO mice had a decreased proportion of proinflammatory Proteobacteria than MNCO mice (p = 0.008). Despite the reduced inflammatory responses in the MNFO mice, they did not have increased susceptibility to the intestinal pathogen *Citrobacter rodentium*. Collectively, these data indicate that inclusion of omega-3 PUFAs in nutritional interventions for acute malnutrition can improve impaired intestinal barrier function, reduce bacterial translocation, and blunt the exaggerated inflammatory response without compromising defense against an intestinal pathogen.

1331

THE UTILITY OF TAQMAN ARRAY CARD TECHNOLOGY FOR DETERMINATION OF THE CAUSE OF DEATH IN CHILDREN UNDER 5 YEARS OF AGE IN WESTERN KENYA

Frederick Ade[1], Jim Katieno[1], Victor Akelo[2], Revathi Gunturu[3], Maureen Diaz[4], Jonas Winchell[4], Dianna Blau[4], Elizabeth Hunsperger[2], Dickens Onyango[5], Beth A. Tippett Barr[2], **Clayton Onyango**[2]

[1]*Kenya Medical Research Institute, Kisumu, Kenya,* [2]*Centers for Disease Control and Prevention, Kisumu, Kenya,* [3]*Aga Khan University Hospital, Nairobi, Kenya,* [4]*US Centers for Disease Control and Prevention, Atlanta, GA, United States,* [5]*Kisumu County Department of Health, Kisumu, Kenya*

Despite reductions over the past two decades, childhood mortality remains high in low income settings in sub-Saharan Africa and south Asia. Many health facilities in sub-Saharan Africa are unable to determine specific cause of death due to minimal access to laboratory services while the patient is alive, and the lack of post-mortem services, complicated further by low cultural acceptability of autopsies. The Child Health and Mortality Prevention Surveillance (CHAMPS) study used three separate TaqMan Array Cards (TAC) on each case investigated. TAC is a multi-pathogen PCR-based detection method for laboratory diagnosis in deceased children under 5years. Combined with minimally invasive techniques to collect samples, TAC provided results to aid in an expert panel's determination of cause of death. Total nucleic acids (TNA) were extracted from 400 µl of cerebral spinal fluid, blood, homogenized lung tissue and 350 µl nasopharyngeal fluid using QIAamp DNA mini kit. Stool material (400 µl) was extracted using QIAamp Fast Stool mini kit and nucleic acids (NA) eluted in 100µl elution buffer for all sample types. Different TAC assays were set up using 50 µl NA in a total reaction of 100µl, card loaded in ViiA7 thermal cycler, and run files analysed using Quant studio ver 1.2. In the 185 cases investigated, 114 (61.6%) were found postive for infectious agent(s). The pathogens associated with cause of death included *Klebsiella pneumoniae* (12.5%, n=23), *Plasmodium falciparum* (11.4 %, n=21), *Streptococcus pneumoniae* (13.9%, 22), *E. coli/Shigella* (8.1%, n=15), *Haemophilus influenza*e (4.9%, n=9), Adenovirus (4.3%, n=8), Cytomegalovirus (4.3%, n=8), *Staphylococcus aureus* (4.3%, n=8), and Enterovirus (2.7%, n=5). TAC is a broad testing platform for multiple pathogens by syndromes that, combined with clinical presentation, was used in the final determination of death. These results alongside clinical records, clinical laboratory results and verbal autopsy was used by a team of specialists to bring definitive answers to mortality cases investigated in CHAMPS.

1332

YELLOW FEVER VACCINATION: STRATEGIES DURING SHORTAGES AND THE EFFECT OF COVID-19

Lin H. Chen[1], Mary E. Wilson[2]

[1]*Mount Auburn Hospital, Cambridge, MA, United States,* [2]*Harvard Chan School of Public Health and UCSF School of Medicine, Boston, MA, United States*

Yellow fever (YF) outbreaks continue, affect new areas, and threaten large populations in South America and Africa. The WHO Eliminate Yellow Fever Epidemics strategies include: expand YF vaccine use, prevent international spread, and contain outbreaks rapidly. Vaccination is the mainstay in prevention and control, but global vaccine supply is often insufficient. COVID-19 impacted all vaccination programs including YF vaccination for travelers. A PubMed search on "yellow fever control" AND "yellow fever vaccine" identified 497 articles published July 2009 through July 2019; articles relevant to YF vaccine supply/shortages were selected, reviewed, and summarized. Sanofi Pasteur US supplied data on YF vaccines distributed 2017-2020. During outbreaks that led to YF vaccine shortages, fractional-dosing was the primary dose-sparing response. Outbreaks (2015-2016) in Angola and Democratic Republic of the Congo (DRC) necessitated fractional-dosing in DRC; 98% seropositivity was achieved at 28-35 days post-vaccination, effectively controlling outbreaks. Brazil has been using fractional-dosing since 2018 to protect populations. In non-endemic countries with vaccine shortage, responses included using non-FDA licensed vaccine (Stamaril™) through Expanded Access Investigational New Drug Program (EAP,US) or investigator-initiated clinical trial (Japan), and YF-VAX™ fractional-dosing (Canada). By March 6, 2020, the US EAP registered 639,850 doses shipped but fell rapidly. Another strategy, intradermal administration, showed 91% achieving 90% virus neutralization 4 weeks post-vaccination, but need further evaluation before wider adoption. The current global YF vaccine supply is often inadequate to meet expanded demand. Vaccine shortages have occurred due to YF outbreaks, manufacturing issues, and distribution problems. Off-label uses of vaccine have been used to meet demand during outbreaks. These strategies provide near-term approaches to protect populations while seeking ways to avoid future vaccine shortages. The COVID-19 Pandemic dramatically reduced YF vaccinations.

1333

HETEROLOGOUSLY EXPRESSED SLO-1 ISOFORMS FROM *ONCHOCERCA VOLVULUS* AND *O. OCHENGI* EXHIBIT SIMILAR LEVELS OF EMODEPSIDE SENSITIVITY

Steffen R. Hahnel[1], Anouk Sarr[2], Lucien Rufener[2], Iring Heisler[1], Sebastian Schneckener[3], Benjamin Makepeace[4], Martin Glenschek-Sieberth[5], **Daniel Kulke**[1]

[1]*Bayer Animal Health GmbH, Leverkusen, Germany,* [2]*INVENesis, Neuchâtel, Switzerland,* [3]*Bayer AG, Leverkusen, Germany,* [4]*University of Liverpool, Liverpool, United Kingdom,* [5]*Bayer AG - Division Pharmaceuticals, Leverkusen, Germany*

The current strategy to control onchocerciasis (river blindness) relies on mass drug administration using ivermectin, which shows robust efficacy against *Onchocerca volvulus* microfilariae but has little impact on adult worm lifespan. Thus, a macrofilaricidal drug is urgently needed to effectively cure patients and sustainably disrupt the parasite's lifecycle. The anthelmintic drug emodepside is currently being developed by Drugs for Neglected Diseases initiative and Bayer AG as a macrofilaricide against *O. volvulus*. As part of the preclinical package, the efficacy of emodepside was evaluated in the closely related cattle parasite *O. ochengi.* To validate the predictability of emodepside effects in the cattle model for human onchocerciasis, we compared the voltage-gated potassium channel SLO-1 of both species, which is widely accepted to be the major drug target of emodepside. Using *O. ochengi* RNA we were able to identify two SLO-1 isoforms and confirm their expression in macrofilariae. Sequence alignments with the corresponding *O. volvulus* isoforms revealed that SLO-1 is highly conserved between both species. To investigate potential species- and isoform-specific differences of SLO-1, we expressed the two identified *O. ochengi* isoforms as well as five *O. volvulus* isoforms heterologously in *Xenopus laevis* oocytes for functional characterization. We demonstrated by electrophysiological recordings of emodepside-concentration-dependent currents that all SLO-1 isoforms formed functional homomeric channels in the oocytes. The analyses of EC_{50} values showed no significant difference of any of the homomeric channels, indicating similar levels of emodepside sensitivity between both species as well as isoforms. In summary, the molecular and pharmacological data presented herein strongly support the predictability of the *O. ochengi* cattle study for anthelmintic efficacy of emodepside against *O. volvulus* and suggest that target-based resistance is unlikely to occur by shifts in isoform-specific expression pattern.

1334

FUNCTIONAL PROFILING OF G PROTEIN-COUPLED RECEPTORS AS CANDIDATE ANTHELMINTIC TARGETS USING "PARASITIZED" *CAENORHABDITIS ELEGANS*

Kendra Joy Gallo, Nicolas J. Wheeler, Abdifatah M. Elmi, Kathy Vaccaro, Mostafa Zamanian

University Wisconsin-Madison, Madison, WI, United States

Parasitic nematodes infect over 1.5 billion people and disease control efforts rely on mass drug administration (MDA) of a severely limited number of drugs. Sub-optimal anthelmintics and the growing threat of resistance motivates the need for the diversification of drug targeting mechanisms. G protein-coupled receptors (GPCRs) remain unexploited as anthelmintic targets despite their involvement in critical sensory, neuromuscular, and physiological processes. One significant bottleneck in exploring the pharmacology of parasite GPCRs results from difficulties in consistently establishing heterologous expression in single-cell systems. Yeast and mammalian cell culture systems have paved the way for deorphanization of helminth GPCRs, but not all receptors express properly in cell types derived from distant phylogenetic lineages. The combinations of accessory proteins, molecular chaperones, and membrane determinants required for the successful trafficking and signaling of parasite receptors in surrogate systems have not been comprehensively identified. To avoid some of these complications, we've established new endpoints for parasite GPCR expression in the model nematode *C. elegans*. We probe the function and pharmacology of a putative muscarinic acetylcholine receptor (*Bma-gar-3*) from *Brugia malayi*, an etiologic agent of lymphatic filariasis. Receptor expression in the pharynx, body wall and ASH sensory neurons of *C. elegans* can be coupled to numerous phenotypic endpoints of receptor activation. We have optimized a series of plate-based and electropharyngeogram (EPG) assays that allow us to perturb and explore parasite receptor function and pharmacology in a nematode physiologic context. While expression in scalable single-cell systems will remain a worthwhile objective for small-molecule screens against GPCR targets, functional parasite receptor assays in a nematode cell and physiological environment can provide important baseline pharmacological data. Finally, we explore the possibility that assays with "parasitized" *C. elegans* can be ultimately adapted for high-throughput screening.

1335

ASSESSING HOOKWORM RESPONSE TO ALBENDAZOLE TREATMENT ACROSS FIVE REGIONS IN GHANA: TOWARDS THE DEVELOPMENT OF AN ANTHELMINTHIC RESPONSE MAP

Elias K. Asuming-Brempong[1], Yvonne Ashong[2], Jewelna E. Akorli[2], Bright K. Idun[1], Freda K. Boampong[1], Rahmat B. Yussif[2], Rahmat B. Yussif[2], Manfred Asiedu[1], Frank T. Aboagye[1], Michael Cappello[3], Michael D. WIlson[2], Mike Y. Osei-Atweneboana[1]

[1]*Biomedical and Public Health Research Unit, Council for Scientific and Industrial Research-Water Research Institute, Accra, Ghana,* [2]*Noguchi Memorial Institute for Medical Research, Accra, Ghana,* [3]*Yale Pediatric Infectious Diseease, Yale New Haven Hospital, Yale University, New Haven, CT, United States*

Hookworm infection is a major Public Health concern, constituting approximately 26% of the 4.6 billion soil-transmitted helminth infections worldwide. In Ghana, Mass Drug Administration (MDA) of albendazole (ALB; 400mg) has been utilized for the national deworming of schoolchildren since 2007 through the Neglected Tropical Disease Programme of Ghana Health Service. After a decade of annual MDA, there are concerns that poorly responding hookworm phenotypes may be emerging. This study sought to measure ALB effectiveness against hookworm, while providing an updated prevalence/intensity map of hookworm in Ghana. We surveyed 40 communities across 13 districts and 5 regions: Greater Accra (GAR), Volta (VR), Brong Ahafo (BAR), Northern (NR), and Upper East (UER). For each community, 50 households were randomly selected; and two participants (1 adult, 1 child) were recruited from each household. After informed consent, questionnaires were administered and stool samples examined for the presence of soil-transmitted helminth (STH) eggs. Hookworm-positive subjects were treated with ALB (400mg); and follow-up was done 12-13 days after treatment. Baseline prevalences were 0.64% in the GAR, 6.48% in the VR, 12.78% in the BAR, 4.29% in the NR, and 1.63% in the UER. FU prevalences were 0.64%, 1.50%, 3.58%, 0.66%, and 0.16% for the respective regions. Faecal egg count reduction rates (FECRR) were 47.06% in the GAR, 97.32% in the VR, 91.12% in the BAR, 86.83% in NR, and 97.44% in the UER, indicating varied ALB efficacies across study sites. Also, we observed generally higher infection intensities among adult-, compared to child-, participants, a trend which persisted at FU. Hookworm prevalence and intensity were higher in the BAR and VR (Transitional Zone), as compared to the NR and UER (Savanna Zone); and the GAR (Coastal Zone). These findings of higher hookworm prevalence in adults and the variable response to drug treatment are of particular relevance to current control strategies in Ghana, which target exclusively school-aged children (SAC) and rely on single dose ALB for hookworm and other STHs.

1336

PREVALENCE, CLINICAL AND LABORATORY PRESENTATION OF FILARIASIS AMONG INDIVIDUAL SCREENED FOR THE FUTURE MALARIA CLINICAL TRIALIN BIOKO ISLAND, EQUATORIAL GUINEA

Alejandro Bolopa[1], Maria Silvia Lopez[2], Vicente Urbano Nsue Ndong[2], Said Jongo[3], Thabit Mbaga[3], Beltran u Ekua Ntutum[2], Ali Hamad[3], Wonder Philip Phiri[1], Julia Yumbe Baka[1], Ines Toichoa[1], Olivier Tresor[1], Godwin Fuseini[1], Guillermo Garcia[1], Genaro Nsue[2], Juan Carlos Momo[2], Maximillian Mpina[4], Elizabeth Nyakarungu[1], Matilde Riloha Rivas[2], Raul Chuquiyauri[1], Preston Church[5], Peter Billingsley[5], Thomas Richie[5], Stephen L. Hoffman[5], Kamaka Ramadhani Kassim[3], Salim Abdulla[3], Claudia Daubenberger[6]

[1]*Medical Care Development International, Malabo, Equatorial Guinea,* [2]*Ministry of Health and Social Welfare, Malabo, Equatorial Guinea,* [3]*Ifakara Health Institute, Dar es salaam, United Republic of Tanzania,* [4]*Swiss Tropical and Public Health Institute, Basel, United Republic of Tanzania,* [5]*Sanaria Inc, Rockville, MD, United States,* [6]*Swiss Tropical and Public Health Institute, Basel, Switzerland*

Filariasis is a disease group caused by filariae that affects humans. The filariasis typical of man can be classified into two groups; pathogenic filariae and apathogenic or little pathogenic filariae. Adult apathogenic filariae's are located on the skin (*Dracunculus medinensis, Loa loa* and *Onchocerca volvulus*) or in the lymphatic vessels (*Brugia malayi, B. timori* and *Wuchereria bancrofti*) while apathogenic are located on the skin (*Mansonella streptocerca*) or in the serosa (*M. ozzardi* and *M. perstans*). These diseases are major causes of morbidity in many developing countries and affect over 150 million persons worldwide. In Equatorial Guinea, there has been a program for the fight against Onchocerciasis and other filariasis for several years since 1987. However, report revealed the presence of microfilariae in the peripheral blood of patients who attended the hospital for malaria blood slide test in the country. Hence, there is a need to have the clear understanding and updated information of the burden of the filariasis disease in Bioko Norte to monitor the progress of the program. The objective of this study is to evaluate the prevalence, clinical and laboratory presentation of filariasis among individual screened for the future Malaria clinical trial in Bioko Island, Equatorial Guinea.The study design is descriptive cross-sectional study. The data will be obtained from clinical data-set of the study entitled Pilot Study to Optimize Recruitment and Screening Procedures for Future Clinical Trials and to Create a Registry of Potential Research Participants on Bioko Island, Equatorial Guinea. The pilot study is expected to recruit 3500 healthy males and females aged 18 months to 50 years old living in selected areas of Bioko Island with high malaria transmission. Descriptive analysis will be used to scrutinized clinical presentation, hematological and biochemical variables. The statistical analysis will be done by SPSS software. The study started in September 2019 and final monitored data is expected to be available in July 2020.

1337

SUSTAINED *INTERRUPTION* OF *WUCHERERIA BANCROFTI* TRANSMISSION TEN YEARS AFTER STOPPING MASS DRUG TREATMENT WITH ALBENDAZOLE AND IVERMECTIN IN SIKASSO HEALTH DISTRICT, MALI

Moussa Sangare[1], Lamine Diarra[1], Yaya Ibrahim Coulibaly[2], Siaka Yamoussa Coulibaly[3], Housseini Dolo[3], Abdoul Fatao Diabate[3], Yacouba Sanogo[3], Salif Seriba Doumbia[3], Michel Emmanuel Coulibaly[3], Lamine Soumaoro[3], Abdallah Amadou Diallo[3], Thomas Bruce Nutman[4]

[1]. *International Center of Excellence in Research (ICER-Mali), Filariasis Research and Training Unit, Bamako, Mali, Bamako, Mali,* [2]. *International Center of Excellence in Research (ICER-Mali), Filariasis Research and Training Unit, Bamako, Mali/ Dermatology Hospital of Bamako, Bamako, Mali, Bamako, Mali,* [3]*International Center of Excellence in Research (ICER-Mali), Filariasis Research and Training Unit, Bamako, Mali, Bamako, Mali,* [4]*National Institutes of Health, Laboratory of Parasitic Diseases, Helminth immunology Section, Bethesda, Maryland; World Health Organization, Vectors, Environment and Society Research, Geneva, Switzerland, Bethesda, MD, United States*

After seven consecutive annual rounds, mass drug administration (MDA) was stopped in 2008 in six *Wuchereria bancrofti (*Wb*)* hyperendemic villages of Sikasso Health District (HD). In 2009, an evaluation program was established to periodically assess the HD to detect early possible reemergence of the infection. The current study, designed to assess Wb transmission 10 years after MDA cessation was, conducted in the summer of 2019 using FTS (filarial test strip) and the SDBioline Oncho/LF IgG4 biplex in children aged 6-7 years and in adults over 15 years. Thick smears of night blood were performed on all FTS- and/or Wb123-positive subjects. A total of 2,046 participants were tested. The overall prevalence of antigenemia using FTS was 0.46% (1/219) in children and 1.63% (25/1827) in adults. The overall prevalence of IgG4 against Wb123 was 0% (0/219) in children and 0.55% (10/1827) in adults. No *W. bancrofti*

microfilaremia was found among the positive participants. Our data suggest the interruption of *W. bancrofti* transmission has been sustained for 10 years after MDA has stopped this HD.

1338

FEASIBILITY OF ROUTINE HEALTH ACTIVITIES EMBEDDED SURVEILLANCE STRATEGY FOR LYMPHATIC FILARIASIS IN THE EVALUATION UNIT OF BOUGOUNI AND YANFOLILA IN MALI

André Vital Dembélé[1], Yaya Ibrahim Coulibaly[2], Housseini Dolo[2], Massitan Dembélé[3], Siaka Yamoussa Coulibaly[4], Salif Seriba Doumbia[4], Moussa Sangare[4], Ilo Dicko[4], Lamine Soumaoro[4], Hamadoun Sangho[1], Seydou Doumbia[1]

[1]*Department of Teaching and Research in Public Health and Specialties, Faculty of Medicine and OdontoStomatology, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali, Bamako, Mali,* [2]*Department of Teaching and Research in Public Health and Specialties, Faculty of Medicine and OdontoStomatology, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali/ Dermatology Hospital of Bamako, Bamako, Mali, Bamako, Mali,* [3]*National Program for the Elimination of Lymphatic Filariasis, Directorate General of Health and Public Hygiene, Ministry of Health, Bamako, Mali, Bamako, Mali,* [4]*Filariasis Research and Training Unit, Faculty of Medicine and OdontoStomatology, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali, Bamako, Mali*

The health districts (HDs) of Bougouni and Yanfolila was the first evaluation unit (EU) to initiate the mass drug administration (MDA) in 2005 because they had the highest LF prevalence in the region. MDA was stopped in 2011 in this HD after a transmission assessment survey (TAS). Ten years after MDA cessation, the present study was initiated to assess the feasibility of an alternative post MDA surveillance strategy integrated into daily routine activities in the health facilities. We conducted a cross-sectional study from November 1, 2018 to April 30, 2019 with convenience sampling design. District health workers were trained to collect data using SDBioline Oncho/LF IgG4 Biplex and the FTS (filarial test trip) rapid diagnosis tests (RDTs). A total of 50 tests per community health center and an average of 192 tests per HD were distributed. Participants included patients who came for routine medical examination or other medical needs requiring blood sample collection in Bougouni and Yanfolila HD's facilities. We performed nighttime blood thick smear to look for microfilaremia for those with positive RDT. Despite a low number of tests available per health facilities, all the tests were used within less than 6 month period. Health staffs were comfortable with performing the tests after the one day training they underwent. The proportion of card lost was < 1 %. Communicating the results was easy through a supervision visit that collected regularly the available results. Participant's median age was 30 (2-100) with a sex ratio of 0.84. In Bougouni, 38/1349 tested and 4/463 tested were children aged 6-7 years respectively with the Biplex and the FTS. In Yanfolila, 14/676 tested and 6/331tested were children aged 6-7 years respectively with Biplex and FTS. All these children were negative for both tests. Positive volunteers were found a microfilaremic. Focusing the sampling on this age group and increasing the number of tests and period of surveillance may provide with a LF surveillance strategy more suitable, sustainable, cheaper and geographically representative of the EU.

1339

THE CHANGING EPIDEMIOLOGY OF LYMPHATIC FILARIASIS AND SOIL-TRANSMITTED HELMINTHIASIS WITH PREVENTIVE CHEMOTHERAPY AND THE FEASIBILITY OF COMBINING SKIN-NTDS WITH THE TRANSMISSION ASSESSMENT SURVEY

Josefina Clarinha João[1], Endang da Silva[1], **Sung Hye Kim**[2], Hanna Jin[2], Younghee Jung[3], Louise Kelly-Hope[4]

[1]*Ministry of Health, Dili, Timor-Leste,* [2]*Hanyang University College of Medicine, Seoul, Republic of Korea,* [3]*World Health Organization, Dili, Timor-Leste,* [4]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom*

Lymphatic filariasis (LF) is a mosquito-borne neglected tropical disease (NTD) targeted for global elimination, primarily by interrupting transmission with preventive chemotherapy through mass drug administration (MDA) to populations at risk in endemic areas. Timor-Leste is endemic for many NTDs, including LF and the national NTD programme has been implementing MDA using albendazole, diethlycarbamazine (DEC) since 2015 and the addition of ivermectin since 2019. Recent surveys found that MDA coverage was high and that LF prevalence (circulating filarial antigen of *Wuchereria bancrofti* and antibody of *Brugia* spp. species) had reduced significantly. It is likely that the LF MDA activities including albendazole, have also reduced the wide prevalence of soil-transmitted helminthiasis (STH).This main aim of this study is use the WHO Transmission Assessment Survey (TAS) protocol to assess the impact of MDA on the prevalence of LF and STH in school children. A secondary aim is to use the TAS platform to screen for skin-NTDs. A cross-sectional observational study is planned for mid-2020 and will i) assess whether multiple rounds of LF MDA have led to the reduction in prevalence to a level where transmission can no longer be sustained ii) assess the epidemiologic profile of STH in selected districts to help inform future decisions on the mass deworming programme and iii) quantify the prevalence of scabies and yaws. In total, seven evaluation units (EUs) including 274 schools will be assessed by testing grade 1 and 2 children for LF CFA using Filariasis Test Strip (FTS), and antibody, using Brugia Rapid and for scabies by clinical assessment and yaws by serology. For STH, two EUs will be assessed by collecting stool amination of egg-producing worm infections by faecal examination by Kato-Katz. Prevalence levels of all NTDs will be calculated, mapped and compared to baseline measures to assess the changes in the epidemiology. The findings from this study will be presented and form the basis for how future NTD intervention and surveillance strategies will be directed and if MDA can be stopped or needs to be resumed for LF and/or STH.

1340

SINGLE-MICROFILARIA GENOTYPING AND KINSHIP RECONSTRUCTION IN *ONCHOCERCA VOLVULUS* FOR DETECTING MACROFILARICIDAL TREATMENT EFFICACY

Tristan P.W. Dennis[1], William Sands[1], Jacob Thompson[1], Millicent Opoku[2], Linda Batsah Debrah[3], Alexander Yaw Debrah[3], Jubin Osei-Mensah[4], Eileen Devaney[1], Michael P. Barrett[1], Ivan Scandale[5], Sabine Specht[5], Lisa Ranford-Cartwright[1], Poppy H.L. Lamberton[1]

[1]*University of Glasgow, Glasgow, United Kingdom,* [2]*Noguchi Memorial Institute for Medical Research, Legon, Ghana,* [3]*Kwame Nkrumah University of Science and Technology, Kumasi, Ghana,* [4]*Kumasi Centre for Collaborative Research in Tropical Medicine, Kumasi, Ghana,* [5]*Drugs for Neglected Disease Initiative (DNDi), Geneva, Switzerland*

Onchocerca volvulus is a nematode parasite, and the causative agent of onchocerciasis, a disease with a devastating and disproportionate impact on vulnerable populations. Community Directed Treatment with Ivermectin (CDTI) is the current mainstay of control but despite decades of CDTI, reports of high disease burden, transmission and recrudescence are commonplace, necessitating the development of new macrofilaricides. Evaluation of treatment outcome is currently conducted via skin snips and microfilariae counts, and nodule excision and histology of adult parasites. Only a proportion of nodules are accessible, and surgery to excise them

is invasive, difficult to conduct on a large scale, and itself constitutes a form of treatment, disrupting accurate measurement of trial outcomes. The genotypes of microfilariae, derived from skin-snip biopsies, present a possible alternative marker, that can be used to determine whether female worms remaining after treatment are the result of reinfection or treatment failure. In this study, we performed single-microfilaria and adult female whole-genome-sequencing on O. volvulus samples derived from ten patients from Ghana. Combining skin-snip and intrauterine microfilariae, and adult female genotype data we provide a preliminary estimate of the relative contribution of female worms to the total microfilarial burden. We provide proof-of-principle that offspring genotypes can be used both to estimate adult female genotypes, and as a proxy for genotyping adults as a marker of treatment failure or success. We compare the use of mitochondrial sequencing for reconstructing unique maternal haplotypes, and whole-genome data to reconstruct kinships between microfilariae, as a proxy for adult sampling. We will discuss the both approaches: their ease of use, cost, utility in detecting maternal genotypes and kinships, and their ability to be used in statistical models to infer total adult worm burdens. These data form the basis of a workflow that can be deployed in clinical trials for macrofilaricides and generalised to other human and animal macroparasites.

1341

MOLECULAR CHARACTERIZATION OF *WUCHERERIA BANCROFTI* FROM MICROFILARAEMIC INDIVIDUALS IN NEPAL

Shyam P. Dumre[1], Ram Kumar Adhikari[2], Kamal Ranabhat[2], Renu Bhandari Dumre[3], SL Hoti[4], Jeevan Bahadur Sherchand[2], Rajendra Raj Wagle[2]

[1]*Kantipur College of Medical Science, Kathmandu, Nepal,* [2]*Institute of Medicine, Tribhuvan University, Maharajgunj Medical Campus, Kathmandu, Nepal,* [3]*School of Tropical Medicine and Global Health, Nagaski University, Nagasaki, Japan,* [4]*Indian Council of Medical Research, Karnataka, India*

Nepal has made substantial progress towards the elimination goal of lymphatic filariasis (LF) with the introduction of mass drug administration (MDA). Half of the country's districts have qualified to stop MDA and rigorous transmission assessment surveys (TAS) are ongoing. Potential movement of *Wuchereria bancrofti* strains across Nepal-India borders might pose a challenge to elimination programs. No molecular information on *W. bancrofti* is available from Nepal. Therefore, this study attempted to address the lack of genetic information of circulating strains required to understand parasite movements and genetic/strain variability. *W. bancrofti* was recovered from blood samples of microfilaraemic individuals from LF endemic districts of Nepal. *W. bancrofti* abundant larval transcript (*alt-2*) fragment and internal transcribed spacer (*its*) region of 18S ribosomal DNA were PCR-amplified from gDNA and sequenced. The resulting sequences were analyzed for polymorphism in the 29 bp Short Tandem Repeats (STR) in intron-1 of *alt-2* gene and haplotype mapping of the *its* region. Phylogenetic trees were constructed for evolutionary relationships of the circulating strains in Nepal and India.Over hundred polymorphic sites were recognized in the *its* sequence (58 in Nepal alone) indicating strain variation. STR analysis of *alt-2* revealed two distinct polymorphism patterns in Nepal and India. Parasite strains were largely mixed up within Nepal (different regions) and also between two countries as evidenced by phylogenetic trees of *its* and *alt-2* sequences suggesting potential movement of parasites. *Alt-2* phylogenetics showed three distinct lineages/clades of *W. bancrofti* in Nepal. Such genetic comparisons were lacking from Nepal.To conclude, *W. bancrofti* strains circulating between Nepal and India are closely related with potential cross-border parasite movement. This information has important implications in LF elimination since they share open border. Therefore, monitoring by molecular tools should be considered in countries embarking LF elimination.

1342

SUSTAINING INTERRUPTION OF TRANSMISSION FOR LYMPHATIC FILARIASIS IN EIGHT DISTRICTS IN SIERRA LEONE

Abdulai Conteh[1], Ibrahim Kargbo Labour[1], Victoria Redwood-Sawyer[2], Alhassan Konneh[2], Abdulai Koroma[2], Unidiatu Kabia[2], Amy Veinoglou[3], Mustapha Sonnie[2], Mary Hodges[2], Yaobi Zhang[4]

[1]*Neglected Tropical Diseases Program, Ministry of Health and Sanitation, Freetown, Sierra Leone,* [2]*Helen Keller International, Freetown, Sierra Leone,* [3]*Helen Keller International, New York, NY, United States,* [4]*Helen Keller International, Regional Office for Africa, Dakar, Senegal*

All 14 health districts (HDs) of Sierra Leone were endemic with lymphatic filariasis (LF) and after 7 rounds of mass drug administration (MDA), 8 HDs in 4 evaluation units (EUs) successfully passed transmission assessment survey (TAS1) and stopped MDA. In 2019, post-MDA surveillance survey (TAS2) was conducted using the Filariasis Test Strip (FTS). The objective was to determine the prevalence of *W. bancrofti* antigenemia in children 6-7 years of age to demonstrate whether interruption of transmission was sustained in these districts. The TAS survey sample builder was used to determine sample sizes and survey design. As the school enrollment rate was <75% in 2 EUs (Bo and Pujehun, Kono and Tonkolili) the community-based survey was implemented in 81 clusters. As the school enrollment rate was >75% in the other 2 EUs (Port Loko and Kambia, Bonthe and Moyamba) a school-based survey was implemented in 67 schools. A total of 9,007 children aged 6-7 years, were sampled (M: 49.7%, F: 50.3%) from total of 148 clusters and one FTS positive case was found in Kono. This child was retested, confirmed positive and treated with ivermectin and albendazole. The critical cut off value was 20 in each EU and thus all 4 EUs (8 HDs) passed TAS2. These 4 EUs will continue post-MDA surveillance TAS3 in 2021. The capacity of the LF surveillance will be strengthened. Refresher/training will be conducted for district laboratory technicians on the use of FTS for routine checks on patients visiting the hospitals or health centers for blood tests. Furthermore, information, education and communication messages will be modified to transition from a public perception of disease-risk to integrated approach of disease-prevention thereby developing rapid, inexpensive and easy-to-use procedures. Finally, the national Neglected Tropical Disease Program will develop a sustainability plan to maintain elimination by strengthening an integrated monitoring and evaluation system. This will combine both integrated and disease-specific strategies in addition to an investment plan for the Ministry of Finance to understand the cost-benefit of this process.

1343

IMPROVING MASS DRUG ADMINISTRATION COVERAGE FOR LYMPHATIC FILARIASIS IN 2019 BASED ON SUB-DISTRICT LEVEL DATA ANALYSIS

Ibrahim Kargbo Labour[1], Abdulai Conteh[1], Brian Fuller[2], Habib L. Kamara[3], Victoria Redwood-Sawyer[3], Gandi Kallon[3], **Mustapha Sonnie**[3], Amy Veinoglou[2], Mary Hodges[3]

[1]*Neglected Tropical Diseases Program, Ministry of Health and Sanitation, Freetown, Sierra Leone,* [2]*Helen Keller International, New York, NY, United States,* [3]*Helen Keller International, Freetown, Sierra Leone*

Since 2008, annual lymphatic filariasis mass drug administration (MDA) has achieved ≥65% epidemiological coverage (EC) at district-level. However, four rural districts (Bombali, Kailahun, Kenema and Koinadugu) failed a pre-Transmission Assessment Survey (pre-TAS) in 2013 and re-pre-TAS in 2017. The Western Area Rural (WAR) failed pre-TAS in 2017. To identify problematic areas for targeted supervision, subdistrict-level data were analyzed. Subdistrict-level data from 4 rural health districts (HDs) was collated in 2018 including pre-TAS results, sub-district EC, prior coverage survey results, independent monitoring data, and qualitative knowledge of key stakeholders in the HDs. In the rapidly growing WAR, the reported primary health unit (PHU)-level coverage data during 2019 MDA were analyzed. The analysis in 4 rural HDs found surprising levels of mobility across communities making interpretation by sex and/or age group challenging. The social dynamics behind the mobility included

transitioning for employment, 'commuting', trading, timber-logging, cattle herding and primary/secondary/tertiary education between and within HDs and neighboring Guinea/Liberia. This analysis identified communities with highest transmission, weakest coverage and greatest need for special attention. In 2019, repeated targeted supervision and support was undertaken in these HDs during MDA and increased 2019 coverage by 13-20% compared to 2018, though 127 of 390 PHUs still reported EC of <65% based on recognized catchment populations. In the rapidly growing WAR, 35 of 60 PHUs initially reported insufficient coverage (EC <65%) via independent monitoring despite the district's sufficient overall coverage of 67.5%. Four mop-up rounds using independent monitors, traditional healers, Maternal and Child Health Aides, community health workers and Helen Keller program officers were each followed by further PHU-level data analysis until finally all PHUs reached 65% EC and district level coverage increased to 86.7%. In conclusion, subdistrict-level data analysis and resulting actions taken increased the treatment coverage.

1344

SUPERVISION OF MASS DRUG ADMINISTRATION FOR LYMPHATIC FILARIASIS AND ONCHOCERCIASIS USING THE SUPERVISORS COVERAGE TOOL IN SIERRA LEONE

Ibrahim Kargbo Labour[1], **Habib I. Kamara**[2], Abdul Conteh[1], Abdulai Koroma[2], Victoria Redwood-Sawyer[2], Mustapha Sonnie[2], Amy Veinoglou[3], Benoit Dembele[4], Mary Hodges[2]

[1]*Neglected Tropical Diseases Program, Ministry of Health and Sanitation, Freetown, Sierra Leone,* [2]*Helen Keller International, Freetown, Sierra Leone,* [3]*Helen Keller International, New York, NY, United States,* [4]*Helen Keller International, Regional Office for Africa, Dakar, Senegal*

Annual mass drug administration (MDA) for the elimination of onchocerciasis (OV) and lymphatic filariasis (LF) with ivermectin (IVM) and albendazole (ALB) has been implemented since 2008. To be effective, MDA must reach at least 65% of total population (80% of the eligible population). The Supervisor's Coverage Tool (SCT) is a rapid, simple, and inexpensive tool endorsed by the World Health Organization to identify and solve problems to improve MDA coverage. SCT was performed in 12 districts (HDs) 2 weeks into MDA, and chiefdoms in 4 LF 'hotspot' HDs and 8 OV HDs were purposefully selected based on the NTD focal persons' knowledge of hard to reach or repeated low coverage areas and divided into supervisory areas (SAs) each consisting of <5000 persons. A total of 66 SAs were randomly drawn from 12 HDs: 49 in 4 LF HDs and 17 in 8 OV HDs. Supervisors with local languages and e-data reporting skills were trained to enumerate all households (HHs) in the SAs, randomly select 20 HHs from each SA and then interview one randomly selected MDA-eligible person in each of the 20HHs. 980 persons (M: 57%, F: 43%) were interviewed in the LF HDs and 84.1% (M: 82%, F: 87%) recalled swallowing IVM and ALB. 340 persons (M: 59.7%, F: 40.3%) were interviewed in the OV HDs and 85.6% (M: 84.7%, F: 86.9%) recalled swallowing IVM. In the LF HDs, 20 SAs reached good coverage (≥19), 24 SAs were 'cannot conclude' (14-18) and 5 SAs had inadequate coverage (≤13). In OV districts. 7 SAs reached good coverage (≥19), 6 SAs were 'cannot conclude' (14-18) and 4 SAs had inadequate coverage (≤13). Reasons for missing treatments were; not offered the drugs: 68, house/community not visited: 60, out of area: 42, did not hear about the campaign: 10, fear of side effects: 9, sick: 5 and other: 9 (stock out, hungry). Also, 88 of 93 adults who recalled having swallowed ALB in 8 OV HDs should have received IVM only. Findings were relayed to the health units responsible for the catchment communities, the district NTD focal persons and the national NTDP for remedial action. Implementation of the SCT was smooth and quickly confirmed where coverage was low and more human resources and logistics were required.

1345

SUSTAINED INTERRUPTION OF LYMPHATIC FILARIASIS TRANSMISSION IN 55 ENDEMIC HEALTH DISTRICTS OF CENTRAL, NORTH, FAR NORTH AND ADAMAOUA REGIONS, CAMEROON

Biholong Benjamin[1], Ebene Clarisse[1], **Georges Nko'Ayissi**[1], Julie Akame[2], Patrick Mbia[2], Carine Fokam[2], Benoit Dembele[3], Steven D. Reid[4], Ismael Teta[2], Yaobi Zhang[3]

[1]*Ministry of Public Health, Yaoundé, Cameroon,* [2]*Helen Keller International, Yaoundé, Cameroon,* [3]*Helen Keller International, Regional Office for Africa, Dakar, Senegal,* [4]*Helen Keller International, New York, NY, United States*

Cameroon is endemic for lymphatic filariasis (LF) in 137 of 189 health districts (HDs). The country aims to eliminate LF as a public health problem and one of the strategies used towards elimination was to conduct annual mass drug administration (MDA), according to the World Health Organization recommendations. By 2018, all endemic HDs successfully received at least 5 effective rounds of MDA and passed pre-transmission assessment survey (pre-TAS). From 2014 to 2018, 136 of 137 HDs met the criteria to stop treatment by passing TAS1, and only the Akwaya HD has not yet been assessed due to insecurity. During 2018-2019, 55 HDs in Central, North, Far- North and Adamaoua regions underwent the first surveillance survey (TAS2), except Kolofata (not assessed due to insecurity). These HDs were grouped into 20 evaluation units (EUs) according to their epidemiological profile and geographical locations. The Survey Sample Builder (SSB) was used to calculate sample size and select the clusters. The sampled population consisted of children aged 6-7 years. The survey was conducted in communities in the four regions. The Filariasis Test Strip (FTS) was used to detect LF antigen. Data were captured on smartphones using (open data kit) ODK technology, stored on an ONA platform and processed through an electronic template with control measures. The teams performed day-time calibrated blood smears (CBS) in *Loa Loa* co-endemic areas. Children testing positive were all confirmed by a second FTS test. 33,314 children in 658 clusters were tested and 9 children were confirmed positive. The number of positive children in each EU ranged from 0 to 5, all below the critical cut-off value. The TAS2 results confirmed the sustained interruption of the LF transmission in these 55 HDs. Subsequently, surveillance activities must continue to contribute to the preparation of the LF elimination dossier in Cameroon.

1346

PROGRESS TOWARDS ONCHOCERCIASIS ELIMINATION IN TANZANIA: METHODS AND RESULTS OF MONITORING TRANSMISSION OF *ONCHOCERCA VOLVULUS* IN 16 ENDEMIC DISTRICTS

Oscar Christian Kaitaba[1], Upendo Mwingira[2], Akili Kalinga[3], Dennis Dobbies[4], Kim Won[5], Andreas Nshala[4], kathryn Crowley[2], Veronica Kabona[4], George Kabona[1]

[1]*Ministry of health, Dodoma, United Republic of Tanzania,* [2]*RTI, Washington, DC, United States,* [3]*National Institute for medical Research, Dar es salaam, United Republic of Tanzania,* [4]*IMAworldhealth, Dar es salaam, United Republic of Tanzania,* [5]*CDC, Atlanta, GA, United States*

Tanzania is endemic with Onchocerciasis in 28 (15.1%) districts across seven regions. Following several studies reporting the possibility of eliminating *O. volvulus* through mass drug administration (MDA) at over 80% treatment coverage, various countries including Tanzania ,shifted from a control to an elimination strategy as advocated by World Health Organization (WHO). This shift was in line with the national and global objectives and aimed to achieve elimination by 2025.WHO recommends monitoring of infection every after four to five rounds of MDA to check progress towards elimination by demonstrating a <0.1% seroprevalence of OV-16 antibody using ELISA in children ages 5-9 years. Community-based cross-sectional monitoring surveys were conducted to estimate the prevalence of *O. volvulus* among children below 10 years of age in 16 districts. in October 2018. In each district, first line villages were identified

based on availability of potential breeding sites and presence of biting activity of vectors as determined by the entomological team. All hamlets in the selected first line village were studied. At least 70 households as determined by at least two children of specified age in each household (national Bureau of statistics) were selected using Systematic sampling technique in all hamlets to give at least 110 children in each first line village .All children aged 5 to 9 years old in the selected households were studied to make a minimum of 300 children from the 3 selected villages in district. A total of 5,323 Dried Blood Spots (BDS) samples were collected across the 16 study districts and OV-16 ELISA The study outcomes will help in learning, programmatic planning and decisions.

1347

USE OF SUPERVISOR'S COVERAGE TOOL TO IMPROVE QUALITY AND COVERAGE FOR LYMPHATIC FILARIASIS MASS DRUG ADMINISTRATION IN AGADEZ REGION, NIGER

Salissou Adamou[1], Sita Hamadou[2], Youssouf Yaye[2], Hadiara Adamou[3], Mounkaila Issoufou[2], Ramatou Tourawa[1], Kadi Hassane Diop[1], Benoit Dembele[4], Amy Veinoglou[3], Josette Vignon[2], Yaobi Zhang[4]

[1]*National Onchocerciasis and Lymphatic Filariasis Program, Ministry of Public Health, Niamey, Niger,* [2]*Helen Keller International, Niamey, Niger,* [3]*Helen Keller International, New York, NY, United States,* [4]*Helen Keller International, Regional Office for Africa, Dakar, Senegal*

Niger has conducted annual mass drug administration (MDA) since 2007 and has made progress toward eliminating lymphatic filariasis (LF) as a public health problem: 43 of 54 endemic health districts (HD) have reached the criteria to stop MDA. In Dec 2019, Niger implemented LF MDA in two HDs (Arlit and Iférouane) in Agadez region, where coverage was historically low due to insecurity, frequent population movement, vast geography and difficult terrain. The supervisor's coverage tool (SCT), a practical and cost-effective tool endorsed by WHO to identify and solve problems to improve MDA coverage, was implemented in the two HDs during LF MDA. All health areas were split into supervisory areas (SAs) depending on population sizes and at least one SA was selected each health area. Twenty households were randomly selected per SA and one person from each of the 20 households were interviewed. SCT training was integrated into the MDA cascade training. The tool was implemented by local supervisors with support from district, regional and central level supervisors. The coverage was categorized as "good", "inadequate" or "cannot conclude". As a result, coverage during SCT was good in 9 of the 11 supervision areas (SA) in Iférouane. In Arlit, coverage was good in 13 SAs, poor in 3 SAs (≤10) and inconclusive in 4 SAs (11-15) of the 20 SAs. Main reasons for missing MDA were CDDs did not come (37.7%), unaware of MDA (32.5%) and fear of side effects (6.5%). Supervisors organized daily debriefings and developed specific action plans in low coverage areas, including increased social mobilization, mop-up, redeployment of CDDs from areas with good coverage and reinforcement of proximity supervision. The two HDs ultimately reported epidemiological coverage of 78% and 82% respectively. In addition, program found that SCT implementation by the health center supervisory staff strengthened quality of supervision and made the reported data more credible to them. Moreover, SCT increased ownership and accountability of the MDA campaign among the local health staff and helped raised awareness and commitment at all levels toward the goal of increasing MDA coverage.

1348

ONCHOCERCIASIS VECTORS AND TRANSMISSION STATUS OF *ONCHOCERCA VOLVULUS* AFTER 17 YEARS OF MASS DRUG ADMINISTRATION WITH IVERMECTIN IN MUHEZA DISTRICT, TANGA FOCUS IN TANZANIA

Akili Kalinga[1], Deus Ishengoma[1], Oscar Kaitaba[2], Andreas Nshala[3], Denis Kailembo[3], Katherine Gass[4], Kimberly Won[5], Seth Misago[6], Paul Martin[6], Victoria Lorry[7], Upendo Mwingira[8]

[1]*National Institute for Medical Research, Dar es Salaam, United Republic of Tanzania,* [2]*Ministry of Health, Community Development, Gender, Elderly and Children, Dar es Salaam, United Republic of Tanzania,* [3]*IMA World Health, Dar es Salaam, United Republic of Tanzania,* [4]*NTD Support Center, Task Force for Global Health, Decatur, GA, United States,* [5]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [6]*National Institute for Medical Research, Tanga Centre, Tanga, United Republic of Tanzania,* [7]*National Institute for Medical Research, Tukuyu, United Republic of Tanzania,* [8]*RTI International, Washington, DC, United States*

After over 17 years of annual mass drug administration (MDA) with average therapeutic coverage around 80% through community directed treatment with ivermectin (CDTI), it was envisaged that transmission would be interrupted to allow progress toward elimination of onchocerciasis in Tanzania. A cross-sectional study was conducted in six villages distributed in six wards each in Muheza district in Tanga focus to evaluate the entomological status to understand the impact of CDTI on elimination of *Onchocerca volvulus* infection in the vectors. Black flies were captured using a standard method of human landing collection from June 2017 through August 2017, a peak period for biting activity and transmission of the parasite. Biting rate was calculated by dividing total flies collected by number of capture days. Collected flies were morphologically identified macroscopically, confirmed microscopically and preserved in absolute ethanol in pools of 100 flies by catching point. Polymerase chain reaction (PCR) using O-150 pool-screening method was performed in 2019 to detect *O. volvulus* infection in black flies. A total of 5,119 adult female black flies were collected at six catching points for 24 days. *Simulium woodi* were the main vector collected in the study area with daily biting rate of 213 flies/day. A total of 54 pools of heads and bodies of black flies were analyzed and 3 (5.6%) pools tested positive for *O. volvulus* which is over 0.1%, the current threshold for stopping MDA. Each positive pool came from a separate catching point in the study area, hence 3 of 6 (50%) sites had a positive signal. These findings suggest that despite long term MDA with ivermectin in Muheza district, transmission of *O. volvulus* is ongoing in vectors and humans.

1350

ONCHOCERCIASIS ELIMINATION MAPPING: PREVALENCE OF OV-16 IN IVERMECTIN NAÏVE AREAS OF NORTHWESTERN TANZANIA

Dennis Kailembo[1], Andreas Nshala[1], Upendo Mwingira[2], Akili Kalinga[3], Kerry Dobbies[4], Darin Evans[5], Elizabeth Plunkett[2], Abdel Direny[4], George Kabona[6]

[1]*IMAworldhealth, Dar es salaam, United Republic of Tanzania,* [2]*RTI, Washington, DC, United States,* [3]*National Institute for medical Research, Dar es salaam, United Republic of Tanzania,* [4]*IMAworldhealth, Washington, DC, United States,* [5]*USAID, Washington, DC, United States,* [6]*Ministry of health, Dar es salaam, United Republic of Tanzania*

In Tanzania, Onchocerciasis is targeted for elimination by 2025.The world Health Organization (WHO) recommends that Onchocerciasis Elimination Mapping (OEM) is used to properly identify the areas to be treated with MDA for the purpose of elimination of the infection of Onchocerca volvulus (OV). Therefore, the main objective of OEM was to determine the prevalence of *Onchocerca volvulus* in two districts (Karagwe and Kyerwa) located in Kagera region. The districts were selected due to their proximity to known endemic areas across the borders in Rwanda and Uganda. These 2 districts borders known Onchocerciasis endemic areas as previously reported by APOC and ESPEN. .The selected two districts are

LF non endemic and were also not included in the rapid epidemiological mapping (REMO) that used to identify areas for MDA and hence are Ivermectin naïve districts and likely be reservoir of infection ii are not included in in MDA over the course of elimination of OV in Tanzania. A community-based cross-sectional design survey was conducted in these two districts in June 2019. A two staged sampling procedure was used to select Study villages and households were sampled in two stages: firstly, in each district, five first line villages were purposefully selected for inclusion in the study. The first line villages were identified based on availability of potential breeding sites of vectors. Secondly, an additional 20 villages were systematically sampled with probability to population size. All adults 20 years and older living in the selected households were included in the study. A total of 3,062 dried blood spots (DBS) samples were collected across the two study districts and OV-16 ELISA. Lessons learnt from these surveys and programmatic implications will be presented.

1351

LYMPHATIC FILARIASIS AND MALARIA CO-INFECTION IN MOSQUITO VECTORS IN RURAL TANZANIA

Andreas M. Nshala[1], Upendo John Mwingira[2], Cecilia Uisso[3], Billy Ngassala[1], Andreas Marternsson[1], Yahya Derua[4], Bernard Malongo[4], Demetrius Max[4], Demetrius Max[4]

[1]*Uppsala University, Uppsala, Sweden,* [2]*RTI, Washington, DC, United States,* [3]*NTD Control Program, Dar Es Salaam, Tanzania, United Republic of Tanzania,* [4]*National Institute for Medical Research, Tanga, United Republic of Tanzania*

Lymphatic Filariasis (LF) and Malaria are co-endemic in many parts of the tropics as they are both transmitted by mosquitos. In Tanzania, two districts of Lindi and Mafia have high LF infections- baseline prevalence of 46% and 53% respectively and after 15 years of annual ivermectin+ albendazole mass drug administration, prevalence still ranges from 2.4% to 7%. The target for elimination is below 2%. Unfortunately, malaria prevalence is high too. In 2017 more than 11% of children below 5 years of age had malaria in Lindi. A monitoring study was conducted in these communities to determine the prevalence of lymphatic filariasis and malaria infections - in human and vectors. Blood samples were collected and tested for circulating filarial antigen (CFA) using filarial test strips. Mosquito catching was done using CDC light traps and gravid traps. Morphological identification and dissection were done to determine vector species and infective stage in the field laboratory. PCR and ELISA were conducted to determine infection rates in the vectors. A total of 1896 female mosquitoes were collected using CDC light traps, and about a half (58.5%, n=1109) were *Cx. quinquefasciatus.* Of the 528 mosquitoes examined for parity 451 (85.4%) were parous. None of the 451 filarial vectors examined had *W. bancrofti* larvae of any stage. However, of 210 malaria vectors examined for malaria parasites, 3 (1.4%) were found to be infected with sporozoites. All infected mosquitoes were members of the *An. funestus* group. PCR pool screening for *W. bancrofti* revealed that 18 out of the 90 mosquito pools were positive. For both trap types and species, the probability that any one mosquito in the pool was infected with any stage of the *W. bancrofti* parasite was estimated at 1.05%. Using ELISA, 1.04% of the mosquitos had malaria (*Plasmodium falciparum* circumsporozoite protein; Pf-CSP). Infective vectors indicate ongoing transmission, for both LF and malaria. Intensifying vector control activities, including consistent use of LLINs complimentary to routine MDA could accelerate elimination.

1352

OPTIMIZED MOLECULAR XENOMONITORING OF *ONCHOCERCA VOLVULUS,* THE CAUSATIVE AGENT OF RIVER BLINDNESS

Jessica R. Grant, Alicia Williams, Steven A. Williams

Smith College, Northampton, MA, United States

The causative agent of onchocerciasis or river blindness, *Onchocerca volvulus*, is a filarial parasite transmitted via black flies. As prevalence of river blindness decreases due to *Onchocerca* elimination programs, tracking the disease becomes more difficult and more critical; more difficult because fewer people carry the parasites and more critical because the prevalence of the parasite must be low enough to eliminate risk of recrudescence. High throughput PCR testing of the black fly vectors of *O. volvulus* offers a practical way to track the level of infection in a region, but molecular xenomonitoring is not without challenges. The assay needs to be sensitive enough to detect the parasite even when the prevalence is low. At the same time, specificity is crucial; the flies may also be biting non-human animals and picking up closely related parasites, such as the bovine parasite *O. ochengi* which is highly similar to *O. volvulus*. We used a bioinformatics-based method for discovering species-specific repetitive elements for PCR target discovery to confirm that the highly repetitive O-150 repeat is the highest copy number sequence in the *O. volvulus* genome. It is also found in high copy number in the *O. ochengi* genome. We combined available data to find subtle differences between the repeat in the two species and developed a new qPCR assay for *O. volvulus* that is both sensitive and specific. In this study, we compared previously published PCR tests, with our newly developed assay to discern the best method for detecting *O. volvulus* in black flies.

1353

ACCEPTABILITY OF IVERMECTIN, DEC AND ALBENDAZOLE FOLLOWING MASS DRUG ADMINISTRATION IN A TREATMENT-NAÏVE POPULATION IN EAST NEW BRITAIN PROVINCE, PAPUA NEW GUINEA

Alison Krentel[1], Krufinta Bun[2], Benedict Mode[3], Daniel J. Dilliott[1], Charles Thickstun[4], Moses Laman[5], Melinda Susapu[6], Makoto Sekihara[7], Gary J. Weil[8], Christopher L. King[2]

[1]*Bruyere Research Institute, Ottawa, ON, Canada,* [2]*Case Western Reserve University, Cleveland, OH, United States,* [3]*East New Britain Province Health Department, Kokopo, Papua New Guinea,* [4]*University of Ottawa, Ottawa, ON, Canada,* [5]*Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea,* [6]*National Department of Health, Waigani, Papua New Guinea,* [7]*Japan International Cooperation Agency (JICA), Kokopo, Papua New Guinea,* [8]*Washington University, St. Louis, MO, United States*

A triple drug regimen of ivermectin, DEC and albendazole (IDA) was delivered by mass drug administration (MDA) in November 2019 to accelerate lymphatic filariasis (LF) elimination in the treatment-naïve province of East New Britain in Papua New Guinea (population ~400,000). We performed a mixed methods acceptability study in 3 districts in February 2020. The primary outcome measures for the community randomized survey were coverage and mean acceptability score (MAS) based on 9 indicators (range 9-36; acceptability threshold=22.5). 95.7% of survey participants (n=257) reported that they had received and swallowed tablets. MAS was 30.98 ±3.93 (SD) which compared to a MAS of 32.62 ±3.47 (SD) in the IDA safety study in Bogia district, PNG in 2017. A drug deliverer survey of community volunteers, nurses and supervisors (n=76) assessed performance and resilience, using the Connor-Davidson Resilience Scale 25 (25-item measure with scores ranging from 0-100). Survey results showed that drug deliverers had good knowledge of the MDA protocol and adhered to it closely. Deliverers scored lower for indicators related to their ability to complete tasks within the allotted time and their ability to obtain assistance from supervisors. Assessments of resilience were varied, with a mean CD-RISC-25 score of 71.4 ±19.0 (SD). Results from in-depth interviews and focus group discussions indicated that communities appreciated receiving MDA and recognized the importance of eliminating LF. Some were concerned about adverse events, and that was related in part to a single serious adverse event that was later judged to have been unrelated to MDA. Vigilance and rapid responses to AEs will be needed again during the next round of MDA in this province. Other voiced concerns included the number of tablets in the IDA regimen and the use of a dosing pole rather than weight to determine dosing. Respondents also requested more time for education prior to MDA. Taken together, these results indicate that MDA coverage was very high in East New Britain and that community acceptability of IDA was excellent. These results bode well for wider use of IDA to eliminate LF in Papua New Guinea.

1353A

COMPARISON OF DIAGNOSTIC MONITORING TOOL CONVERSION DURING LYMPHATIC FILARIASIS ELIMINATION

Daniel J. Tisch[1], Jeff Kovach[1], Estee Cramer[2], Brooke Mancuso[3], Nelly Sanuku[4], Willie Pomat[5], James Kazura[1], Peter Zimmerman[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]University of Massachusetts, Amherst, MA, United States, [3]Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States, [4]Papua New Guinea Institute for Medical Research, Maprik, Papua New Guinea, [5]Papua New Guinea Institute for Medical Research, Goroka, Papua New Guinea

Monitoring strategies for lymphatic filariasis elimination programs are constrained by the lack of a gold standard to test for infection and infectiousness. Existing monitoring tools exhibit discordant test characteristics and different rates of decay. This study compares annual human markers of lymphatic filariasis infection and transmission in 14 communities of Papua New Guinea during and after 3 rounds of mass drug administration (MDA). Nighttime finger prick blood samples were collected before and after annual MDA from 2015 to 2018. Samples were processed for microscopy to detect circulating microfilaria (MF) and serological markers of infection including Bm14, Og4C3, WB123, and FTS. Conversion and decay rates over time are compared with regression models and tests for diagnostic concordance. During the course of annual MDA, the prevalence of LF detected by each diagnostic are discordant (3.3% to 33.4% positive). However, all diagnostic markers significantly decrease during the treatment periods ($p<0.001$). Following an initial significant decrease after the first MDA, the subsequent annual rate of decrease for prevalence determined by FTS, Og4c3 and WB123 occurred at approximately 3% per year while Bm14 decreased more slowly (-0.8% per year). Discordance of these tests decreased with increasing treatment time. Bm14 produced the greatest number of test-positives while microscopy rates approached zero. Different rates of conversion/decay for these assays are compared in these individuals observed annually during treatment. MDA-stopping decisions based on these different results would produce conflicting conclusion about the impact of MDA in combination with long-lasting insecticide treated bednets provided in this population. These empirical observations are useful for revising elimination monitoring methods and thresholds.

1354

IMMUNOLOGICAL AND EPIDEMIOLOGICAL ASPECTS OF CHAGAS DISEASE IN HONDURAS 2020

Cody J. Malone, Ana L. Sanchez

Brock University, St. Catharines, ON, Canada

American Trypanosomiasis also, known as Chagas Disease is a neglected tropical disease responsible for the highest disease burden of any parasitic disease in the western hemisphere. Chagas Disease currently has no cure or treatment once in the chronic stage and can be potentially fatal. Chagas disease is vector borne and caused by the protozoan parasite *Trypanosoma cruzi*. Chagas disease can also be transmitted vertically, by infected food/water, infected blood transfusions and infected organ transplantations. In terms of Disability-Adjusted Life Years (DALYs) Chagas disease is responsible for the sixth most DALYs (700,000) of the Neglected Tropical Diseases globally. Chagas disease is endemic in Central and South America. Several species from the *Triatominae* family can transmit the parasite (via their faeces) responsible for Chagas disease, this study will focus on *Triatoma dimidiata.* Chagas disease is increasingly becoming an issue in high income countries such the USA, Canada and European nations due to migration from endemic countries. In 2011, Honduras was internationally certified for the interruption of Chagas disease transmission by *Rhodnius prolixus* however, *T. dimidiata,* is known as the non-eliminable vector because, of its wild cycle. The National Chagas program in Honduras dissolved in 2017. The absence of a dedicated national program puts communities at risk for Chagas disease. This leaves communities without adequate surveillance which, can lead to resurgence of Chagas disease. The current study will examine the sero-epidemiological prevalence of Chagas Disease in La Hicaca, Yoro, Honduras, a community where the prevalence have never been studied. The prevalence of *T. cruzi* in *T. dimidiata* within the community will also be examined to assess the potential risk of transmission to humans.

1355

PREVALENCE OF CHAGAS DISEASE IN AT-RISK POPULATIONS IN SAN DIEGO COUNTY

Katherine Promer, Kimberly C. Brouwer, Jair L. de Siqueira-Neto, James McKerrow

UC San Diego Health, San Diego, CA, United States

Cardiac complications of Chagas disease significantly impact quality-adjusted life years (QALY) and health care costs, affecting populations outside endemic Latin America due to chronicity and migration patterns. Our study aims to determine the prevalence of Chagas disease in local at-risk populations in San Diego County. Inclusion criteria for this observational study includes: age at least 18 years, birth or residence of at least 6 months in Latin America, and cardiac disease with conduction abnormality on EKG or systolic heart failure. The questionnaire covers demographics, possible exposures, heart disease risk factors and chronic Chagas disease symptoms. Clinical data abstraction occurs via electronic medical record review. Laboratory analysis starts with *Trypanosoma cruzi* ELISA (Hemagen, ARUP) followed by confirmatory serology at the U.S. Centers for Disease Control (CDC) for positive screens. Thus far, 79 people have participated with a median age of 63. Most come from Mexico, but 12 (15.2%) have lived in Central or South America. Of all participants, 8 (10.1%) resulted positive in serologic screening and 3 (3.8%) confirmed as positive by the CDC. An additional participant carries a presumptive diagnosis of Chagas disease due to high pre-test probability, although unable to provide serum for confirmatory testing due to transition to comfort care, bringing the prevalence up to 5.1%. These confirmed and presumptive positive participants came from Mexico, Guatemala, El Salvador and Brazil. The remaining 4 (5.1%) positive screens had negative confirmatory testing and all came from Mexico. The average screening ELISA value for those diagnosed with Chagas disease, including the one presumptive case, was 6.2 compared to 1.5 for the false positive screens. A value up to 1.0 in this assay is considered negative. These results support recommendations to expand screening in the United States but also highlight the limited specificity of available screening assays. We plan to continue enrollment to achieve a target of 200 participants and perform additional testing on leftover serum to identify possible false negative screens.

1356

METEOROLOGICAL FACTORS ASSOCIATED TO A HIGH PREVALENCE OF LEISHMANIASIS IN NICARAGUA

Santiago Ernesto Hernandez[1], Marissa Anne Rickloff[1], Gerardo Blass[2], Nathanael Stanley[1], Benjamin George Jacob[1]

[1]*University of South Florida, Tampa, FL, United States,* [2]*Universidad Nacional Autonoma de Nicaragua, Managua, Managua, Nicaragua*

Environmental factors, such as temperature, elevation, precipitation, and humidity can create a perfect habitat for the vector and intermediate hosts of the parasite and hence enhance the transmission of the disease. The aim of this study was to apply spatial analytical tools for the identification of explanatory, capture point covariates that may play a role in the high prevalence of CL and MCL in three intervention sites of Nicaragua. The clinical diagnosis was conducted by physicians at health posts, health centers and primary hospitals in Nicaragua. The tissue sample was diagnosed at the National Center of Diagnosis and at the Ministry of Health. Initially sampled, CL and MCL, district, regional geolocations were articulated with Thiessen polygons in ArcMap. Subsequently, the environmental explanatory covariates were employed for generating landscape and meteorological time series models. The empirical data was also regressed employing Poissonian probability models in PROC REG. CL and MCL were reported in 90 municipalities of 153 in Nicaragua. Most of the cases were reported in the North Central (76.54%) and Atlantic (21.63%) regions of the country. The analysis of maximum likelihood parameter estimates for the negative binomial regression model reported a significant Pr > Chi-square value only for the altitude in meters (0.0054). The other regressors that followed a decreasing order of significance were specific humidity, precipitation, mean annual temperature, maximum annual temperature, population density, and minimum annual temperature. ArcGIS and SAS can aid in Leishmaniasis' research efforts by revealing meaningful meteorological and mathematical, cartographic estimators associated with CL or ML prevalence. Future research should focus on incorporating spatial models using non-linear dependent/response variables. It is strongly recommended to carry out a spatial model to determine more specific associations between the occurrence of the disease with other environmental factors, such as slope, vegetation, landscape, and proximity to intermediate hosts.

1357

ANALYSIS OF THE CURRENT EPIDEMIOLOGICAL SITUATION OF CHAGAS DISEASE IN JAPAN

Inés María Iglesias Rodríguez[1], Sachio Miura[2], Takuya Maeda[3], Clara Vásquez Velásquez[4], Sumihisa Honda[5], Satoshi Kaneko[6], Kazuo Imai[7], George Ito[8], Taeko Naruse[4], Kenji Hirayama[4]

[1]*Department of Global Health, School of Tropical Medicine and Global Health, Nagasaki University, Nagasaki, Japan,* [2]*NPO organization, MAIKEN, Tokyo, Japan,* [3]*Department of Microbiology, Saitama Medical University, Saitama, Japan,* [4]*Department of Immunogenetics, Institute of Tropical Medicine (NEKKEN), School of Tropical Medicine and Global Health, Nagasaki University, Nagasaki, Japan,* [5]*Department of Nursing Sciences, Graduate School of Biomedical Science, Nagasaki University, Nagasaki, Japan,* [6]*Department of Ecoepidemiology, Institute of Tropical Medicine (NEKKEN), Nagasaki University, Nagasaki, Japan,* [7]*Department of Infectious Disease and Infection Control, Saitama Medical University, Saitama, Japan,* [8]*Consulate General of Brazil in Japan, Tokyo, Japan*

Japan is one of the non-endemic countries with more estimated cases of Chagas Disease (CD), mostly imported from Latin America (LA), around 300,000 migrants, and less than 1% can access to the diagnosis. Several case reports about CD have been published, but no systematic epidemiological study has been performed in Japan. This study aimed to estimate the prevalence of CD in the present migrant population from LA living in Japan. Also, to analyze the socio-demographic characteristic and risk factors of the participants included in the study.This cross-sectional study involved 299 participants from the Latin American migrant population from March 2019 to March 2020. Data were collected on the opportunities for the migrants to participate in a consultation activity officially held by each country's embassy. After getting informed consent, finger-prick was used to obtain blood samples that were analyzed by 2 serological methods ((Enzyme-Linked Immuno-Sorbent Assay (ELISA) and Indirect Immunofluorescence (IIF)) and the Rapid Diagnostic test (RDT). The participants completed a questionnaire with socio-demographic information and risk factors of CD. To analyze the factors associated with positive cases we used multivariable logistic analysis. The participant's ages ranged between 7 and 77 years old with a mean age of 42.7 (SD±14.03). Brazil has a higher number of participants (41.5%), followed by Bolivia and Peru (33.7% and 17.7%, respectively). Seventy percent of the responders had heard about CD. However, less than 5% tested before. Five out of 299 (1.6%) participants were positive by ELISA, IIF, and RDT, mainly from Santa Cruz, Bolivia. The preliminary prevalence is 1.6, higher than the estimated prevalence (0.75%). This prevalence is higher (5%) if we consider just Bolivians. The Japanese Health system may need to consider the introduction of screening programs in the population at risk.

1358

NON-USE OF HEALTH SERVICES IN ENDEMIC REGIONS FOR CHAGAS DISEASE IN BRAZIL: A MULTILEVEL STUDY

Desireé S. Haikal[1], Renata F. Damasceno[1], Ariela M. Ferreira[1], Samara F. Leite[2], Thallyta M. Vieira[1], Lea C. de Oliveira[3], Antonio L. Ribeiro[4], Ester C. Sabino[5]

[1]*Postgraduate Program in Health Sciences, Universidade Estadual de Montes Claros, Montes Claros, Brazil,* [2]*Hospital Aroldo Tourinho, Member of the SamiTrop Research Nucleus, Montes Claros, Brazil,* [3]*Institute of Tropical Medicine, Universidade de São Paulo, Montes Claros, Brazil,* [4]*Department of Internal Medicine, Universidade Federal de Minas Gerais, Montes Claros, Brazil,* [5]*Sao Paulo-Minas Gerais Neglected Tropical Diseases Research Center for Biomarkers Discover, Institute of Tropical Medicine, Universidade de São Paulo, Montes Claros, Brazil*

Chagas disease (CD) represents the neglected tropical disease with the highest burden of morbidity and mortality in Latin America. In Brazil, the access of people with CD to medical care is still marked by inequity. Thus, this study aims to verify whether there is an influence of contextual characteristics of endemic municipalities in remote regions in the use of health services by people with CD. It is a multilevel study organized based on the theoretical model of Andersen & Davidson. The individual data came from the first follow up of a Brazilian cohort study called SamiTrop, in which 1,709 patients from 21 municipalities endemic for CD participated. Contextual data were collected from official Brazilian government databases at the municipal level (population size, municipal human development index, Gini index, coverage of the Family Health Strategy ...). The dependent variable was the use of health services related to CD in the last 12 months (yes *vs* no). The analysis was carried out by means of multilevel binary logistic regression. The proportion of non-use of health services in the last year among CD patients was 23.5 %. Among the contextual variables, the non-use of health services was influenced only by the population size of the municipalities, being lower in people with CD residing in smaller municipalities (OR = 0.642). Among the individual variables, the probability of not using health services was lower in individuals without functional limitations (OR = 0.624) and in individuals with unaltered NT-proBNP levels (OR = 0.464). The present study showed that the non-use of health services by people with CD is influenced by the population size of the municipalities, even after adjustment for individual factors that express the severity of the disease. The greater coverage of public primary health care services in smaller municipalities seems to have favored the use of health services, however this finding does not inform about the quality of care for people with CD in these municipalities.

1359

INFLUENCE OF THE SOCIAL CONTEXT ON SELF-PERCEPTION OF HEALTH IN INDIVIDUALS WITH CHAGAS DISEASE: A MULTILEVEL STUDY

Desireé S. Haikal[1], Ariela M. Ferreira[1], Renata F. Damasceno[1], Samara F. Leite[2], Thallyta M. Vieira[1], Lea C. de Oliveira[3], Antonio L. Ribeiro[4], Maria C. Nunes[5], Ester C. Sabino[6]

[1]*Postgraduate Program in Health Sciences. Universidade Estadual de Montes Claros., Montes Claros, Brazil,* [2]*Hospital Aroldo Tourinho.Member of the Research Center Sao Paulo-Minas Gerais Neglected Tropical Disease Research Center for Biomarkers Discovers (SamiTrop), Montes Claros, Brazil,* [3]*Institute of Tropical Medicine. Universidade de São Paulo, Sao Paulo, Brazil,* [4]*Department of Internal Medicine. Universidade Federal de Minas Gerais, Belo Horizonte, Brazil,* [5]*Department of Cardiology. Universidade Federal de Minas Gerais., Belo Horizonte, Brazil,* [6]*Institute of Tropical Medicine. Universidade de São Paulo., Sao Paulo, Brazil*

This multilevel study investigated the influence of the contextual and individual factors on the self-perceived of health in individuals with Chagas disease (DC). Individual data from persons with CD (n = 1,513) were obtained from 21 Brazilian municipalities, endemic for this infectious disease and that integrate a cohort study (SaMi-Trop Project). Contextual data from municipalities were collected from government public databases. The dependent variable was *self-perceived of health* (positive *vs.* negative). A hierarchical multilevel logistic regression was performed. Findings from this study showed that 22% of the individuals with DC reported a negative self-perception of health. A lower chance of self-perceiving health negatively was evidenced in individuals with CD who lived in municipalities with a smaller population (OR = 0,6). In the other hand, a greater chance of self-perceiving health negatively was noted in individuals who lived in municipalities with a lower number of doctors *per* inhabitants (OR = 1,5) and municipalities with a higher rate illiteracy (OR = 1,6). Among the individual variables, a greater chance of self-perceiving health negatively was noted in individuals with functional limitations (OR = 2,0), who had altered NT-pro BNP level (OR = 1,9), with arterial hypertension (OR = 1,5), with lower income (OR = 1,5), who lived far from the Basic Health Unit (OR = 2,5), who declared irregular monitoring of the health by primary health care units (OR = 1,7), who did not practice physical activity (OR = 1,8), and who smoked (OR = 2,3). In conclusion, a negative self-perception of health by individuals with CD can be considered high. This aspect seems to be related to unfavorable sociodemographic characteristics and limitations imposed by the difficulty of access these individuals to public basic health services.

1360

OCCURRENCE AND SPATIAL DISTRIBUTION OF TRIATOMINES (HEMIPTERA: REDUVIIDAE) INFECTED WITH *TRYPANOSOMA CRUZI* IN AN ENDEMIC BRAZILIAN MUNICIPALITY

Desireé S. Haikal[1], Maria C. Campos[1], Renata L. Ursine[1], Thainara S. Goncalves[2], Ariela M. Ferreira[1], Mara D. Ribeiro[3], Sandra S. Marinho[4], Lea C. de Oliveira[5], Antonio L. Ribeiro[6], Ester C. Sabino[5], Thallyta M. Vieira[1]

[1]*Postgraduate Program in Health Sciences. Universidade Estadual de Montes Claros., Montes Claros, Brazil,* [2]*Universidade Estadual de Montes Claros., Montes Claros, Brazil,* [3]*Epidemiological Surveillance Service. Municipal Health Department of Montes Claros., Montes Claros, Brazil,* [4]*Zoonosis Control Center of the Municipal Health Department of Montes Claros., Montes Claros, Brazil,* [5]*Institute of Tropical Medicine. Universidade de São Paulo, Sao Paulo, Brazil,* [6]*Department of Internal Medicine. Univerdiade Federal de Minas Gerais, Belo Horizonte, Brazil*

Although Brazil has eradicated *Triatoma infestans*, it has a great diversity of autochthonous triatomines vectors of Chagas disease (CD). The aim of this study was to evaluate the occurrence, natural infection, and distribution of species of triatomines in the urban area from Montes Claros, an endemic municipality for CD in Brazil. Data from Montes Claros Zoonosis Control Center (species, type of capture, stage, gender, and natural infection related to *Trypanosoma cruzi*), from 2009 to 2019, were obtained. The places where the triatomines took place were georeferenced and the spatial distribution analysis was performed using Kernel density estimators (Qgis® version 3.4). According to our findings, 277 triatomines were captured and spontaneously delivered by the residents themselves of the urban area from municipality. The stages of species were as follows: nymphs (n = 29), adults (n = 240), and not-identified stages (n = 8). About 50% of these species were captured in the last three years. The species foul were *Triatoma sordida* (n = 189), *T. pseudomaculata* (n = 25), *T. melanocephala* (n = 18), *Panstrongylus diasi* (n = 9), *P. megistus* (n = 1), *P. geniculatus* (n = 12), *Rhodnus neglectus* (n = 11), *R. prolixus* (n = 1), and not-identified (n = 11). About 50% species were female. The intestinal contents from 206 insects were examined and natural infection with *T. cruzi* was observed (6.8%), notably in adult triatomines from species *T. sordida* (n = 9), *T. pseudomaculata* (n = 3), *P. diasi* (n = 1), and *P. geniculatus* (n = 1). About 90% of these species were captures in masonry residences located in the urban area. Significant clusters of the infected species were observed close to green areas distributed in different regions of the municipality. The occurrence of *T. sordida* species was identified in all regions investigated. This study evidente that *T. cruzi* infection in other species of triatomines may be considered an urban health problem related to CD. In this context, it is necessary to strengthen and improve entomological surveillance actions in both rural and urban areas of Brazil.

1361

THE INTERACTION BETWEEN *TRYPANOSOMA CRUZI* AND *STRONGYLOIDES STERCORALIS* INFECTIONS IN THE CHACO/ YUNGAS REGION

Pedro E. Fleitas[1], Noelia Floridia-Yapur[1], Elvia Nieves[1], Adriana Echazu[1], Paola Vargas[1], Nicolas Caro[1], Favio Crudo[2], Ruben O. Cimino[1], Alejandro J. Krolewiecki[1]

[1]*Instituto de Investigaciones de Enfermedades Tropicales, Universidad Nacional de Salta, Salta, Argentina, Salta, Argentina,* [2]*Asociación para el Desarrollo Sanitario Regional (ADESAR), Buenos Aires, Argentina*

Strongyloides stercoralis is a soil transmitted helminth with a complex life cycle and the ability to reproduce in soil and within the human host. On the other hand, *Trypanosoma cruzi* is a protozoan parasite that causes Chagas disease. Both are Neglected Tropical Diseases (NTDs) endemic in northern Argentina with prevalences up to 48% for *S. stercoralis* and up to 50% for *T. cruz*i in localized communities; based on these prevalences, there is a significant possibility of co-infections. A cross-sectional serological survey was carried out in the province of Salta, Argentina. Samples from 680 individuals of six areas in the Chaco/Yungas regions (with a median age of 11 years) were enrolled in this study. All 680 were diagnosed by serology for *T. cruzi* (ELISA and HAI commercial kits Wiener lab.) and *S. stercoralis* (NIE-ELISA). For each parasite, a multivariate logistic regression analysis was performed, and the adjusted odd ratios (AOR) were calculated, to analyze the possible association between the following variables: sex, age, area of residence, and infection with the other parasite. It turned out that 36% were positive for *S. stercoralis*, 12% were positive for *T. cruzi*, and 3% had mixed infection with both parasites. In addition, it was observed that when the prevalences were categorized by area, *S. stercoralis* decreases proportionally with the increases in the prevalence of T. cruzi. The logistic regression for infection with *S. stercoralis* showed a significant association with age (AOR= 3.14) and a significant variability in AOR between areas. Infections with *T. cruzi* presented a significant association with age (AOR=4.18), sex (AOR=1.68), and three areas of residence. In conclusion, the risk of co-infections with *S. stercoralis* and *T. cruzi* appears as not more frequent than at random; there is however and a negative association in the level of prevalence in the analyzed regions, which warrants further studies to confirm and explain these findings.

1362

EVALUATING A PREDICTIVE ALGORITHM FOR SCREENING CHRONIC CHAGAS INFECTION AMONG PEOPLE LIVING IN RURAL AREAS OF MINAS GERAIS- BRAZIL

Fabio De Rose Ghilardi

São Paulo University, São Paulo, Brazil

A questionnaire-algorithm to improve Chagas diagnostic in rural areas of Brazil Chagas disease (CD) is a tropical neglected disease that causes a huge impact in health care and economics in developing countries from Latin America. In Brazil the burden of the Chagas disease is important among rural communities and the lack of access to health care facilities in rural areas keep many cases undiagnosed. A delay identification of clinical symptoms (as cardiac failure, arrhythmia and gastrointestinal disorders) postpone treatments and impacts quality of life, life expectancy and economy of this people. Based on data from REDS (Retrovirus Epidemiology Donor Study, Sabino et al;2013) which consists in general and structured questionnaires applied to the blood donors and their serologic status for CD. We evaluated a set of variables by logistic regression and we developed a questionnaire to be applied in the field as a diagnostic tool. This diagnostic tool is based on 6 questions (yes or no) and the number of positive answers to each question is related to a higher risk of being infected with Chagas disease. In order to evaluate sensitivity and specificity of our questionnaire and to establish it as a practical tool ready to be used in almost all communities even in shortage of health care facilities we tested it paired with standard serological essay in 3 different rural communities of North Minas Gerais: Espinosa, São Francisco e Porteirinha. At the end 2180 people agreed in taking part of the study, they were submitted to informed consent and then screened using the questionnaire plus were submitted to blood ELISA tests. We did find a strong correlation between having Chagas disease and being a first-degree relative of a person that is known to be Chagas infected. The aim of the diagnostic tool was to elucidate those who should be tested first for Chagas diseases, improving access to health care in rural communities.

1363

TRYPANOSOMA CRUZI INFECTION AMONG RURAL AND URBAN VIRGINIA OPOSSUMS AND NORTH AMERICAN RACCOONS FOUND IN NORTH FLORIDA, USA

Norman L. Beatty[1], Zoe S. White[2], Chanakya R. Bhosale[3], Kristen N. Wilson[2], Sarah E. Maestas[3], Samantha M. Wisely[2]

[1]*University of Florida College of Medicine, Department of Medicine, Division of Infectious Diseases and Global Medicine, Gainesville, FL, United States,* [2]*University of Florida Institute of Food and Agricultural Sciences, Department of Wildlife Ecology and Conservation, Gainesville, FL, United States,* [3]*University of Florida Institute of Food and Agricultural Sciences, Entomology and Nematology Department, Gainesville, FL, United States*

Trypanosoma cruzi, the protozoan parasite recognized to cause Chagas Disease, is known to infect a number of mesomammals in the southern United States, including the Virginia opossum (*Didelphis virginiana*) and North American raccoon (*Procyon lotor*). In Florida, very little is known on what species of wildlife harbor *T. cruzi* despite having naturally occurring triatomine vectors or "kissing bugs" that reside in this region. From April 2019 until January 2020 we conducted a cross-sectional study to assess whether *T. cruzi* DNA could be detected in the blood of both rural and urban populations of opossum (N=69) and raccoon (N=55) in north Florida. We collected approximately 0.1mL of venous blood via Nobuto Blood Filter strip from each animal and extracted the DNA using the Qiagen® Puregene® Blood Kit. Utilizing qualitative PCR methods we then amplified the *T. cruzi*–specific primers TCZ1 and TCZ2 to determine the presence of *T. cruzi* DNA in the blood of each animal. Overall, *T. cruzi* DNA was detected in 17% (N=21/124) of the entire group, with 26% (N=18/69) of opossums and 5% (N=3/55) of raccoons being positive. Among the positive opossums, 78% (N=14/18) were rural-dwelling and 22% (N=4/18) were urban-dwelling, respectively. Among the positive raccoons, 66% (N=2/3) were rural-dwelling and 33% (N=1/3) were urban-dwelling. Interestingly, female opossums were disproportionately more infected than males with 83% (N=15/18) having *T. cruzi* DNA detected in their blood. The enzootic transmission of *T. cruzi* among sylvatic and peridomestic wildlife in Florida is not well described but these preliminary findings suggest that *T. cruzi* infection can be found in both rural- and urban-dwelling opossums and raccoons in our region. More research is being done to determine which unique *T. cruzi* discrete typing units (DTUs) are naturally occurring here in Florida and what impact this infection may have on mammalian wildlife in the southern United States.

1364

VISCERAL LEISHMANIASIS IN PREGNANCY AND VERTICAL TRANSMISSION: A SYSTEMATIC REVIEW OF THE LITERATURE ON THE THERAPEUTIC ORPHANS

Prabin Dahal[1], Sauman Singh-Phulgenda[1], Brittany J. Maguire[1], Philippe J. Guerin[1], Piero L. Olliaro[2]

[1]*Infectious Diseases Data Observatory, Centre for Tropical Medicine & Global Health, University of Oxford, Oxford, United Kingdom,* [2]*Centre for Tropical Medicine & Global Health, University of Oxford, Oxford, United Kingdom*

The occurrence and consequences of Visceral Leishmaniasis (VL) in pregnant women is poorly known. A systematic review of all published literature was undertaken to identify reports of VL in pregnancy by searching five clinical databases: Ovid Medline, Ovid Embase, Cochrane libraries, Global Index Medicus, and Clinicaltrials.gov. Any clinical reports describing the disease in pregnancy or congenital transmission were eligible. The search identified 272 records after deduplication, of which 54 (19.8%) met the inclusion criteria. An additional 16 articles were identified by searching the references of the included articles and the IDDO trials library leading to a total of 70 articles (1928 -2020) describing 460 pregnant women with VL included. A total of 387 (84.1%) were treated, 20 (4.3%) untreated, and treatment status was unclear in 53 (11.5%). Of the treated, liposomal amphotericin B (LAmB) was administered to 232 (59.9%), pentavalent antimony (PA) to 94 (24.1%), amphotericin B deoxycholate to 31 (8.0%), paromomycin to 3 (0.8%), a combination of PA and paromomycin to 11 (2.8%), a combination of PA and LAmB to 4 (1.0%), and the drug name was unclear in 12 (3.1%). 58 (12.6%) women were in the first, 106 (23.0%) in the second, and 86 (18.7%) in the third trimester. Trimester was unclear in 173 (37.6%), and in 37 (8.0%), VL was retrospectively confirmed. During the first trimester, there were 5 (14.7%) miscarriages, 1 (2.9%) premature birth in those treated with LAmB (n=34); and 8 (72.7%) spontaneous abortion in those treated with PA (n=11). During the second trimester, there were 2 (5.3%) miscarriages and 1 (2.6%) premature birth in those treated LAmB (n=38); 16 (35.5%) spontaneous abortion and 1 (2.2%) preterm birth in those treated with PA (n=45). A total of 29 cases of confirmed or suspected vertical transmission were identified. Treatment with LAmB during pregnancy appeared to have a better safety profile on both mother and foetus but results should be interpreted with caution as number of cases were small with poor reporting of data. Promoting a standardised reporting in a pregnancy registry could address the current knowledge gap.

1365

DEVELOPMENT & EVALUATION OF CANDIDATE MARKERS FOR THE DIAGNOSIS OF VISCERAL LEISHMANIASIS.

Samson Muuo Nzou[1], Ann Wanjiru Mwangi[1], Matthew Mutinda Munyao[2], Tony Teya[3], Joanne Jepkemei[3], Caroline Wangui Njoroge[3], Peter Kemboi[3], Esther Muthoni Kinyeru[4], Eberhard Zeyhel[5], Robinson Irweka[6], Satoshi Kaneko[7]

[1]*Kenya Medical Research Institute, Nairobi, Kenya,* [2]*Nagasaki University, Institute of tropical medicine-Kenya Medical Research Institute, Nairobi, Kenya,* [3]*Nagasaki University, institute of tropical medicine-Kenya Medical Research Institute project, Nairobi, Kenya,* [4]*Ministry of health, Nakuru,*

Kenya, [5]Meru University of science and technology, Meru, Kenya, [6]Pan-African University Institute of Basic Sciences, Technology and Innovation, Nairobi, Kenya, [7]Nagasaki University, Japan, Japan

Visceral Leishmaniasis is part of the leishmania complex causing devastating morbidity & fatalities in affected individuals. It is responsible for over 500,000 new cases & 50,000 deaths annually. In Africa, it is caused by *Leishmania donovani (VL) &* actively transmitted by the Phelebotomus sandfly. Splenic aspirate observed under the microscope for amastigote identification is the gold standard for its diagnosis. This is invasive, painful, requires skilled personnel & hospitalization for sample taking. Advances have been made in developing a serological diagnosis test kit, especially with the introduction of the rK39 kit. The kit has brought light in serological diagnosis but suffers in its inability to differentiate between asymptomatic & symptomatic cases, less sensitive & also because the protein is derived from *Leishmania donovani chagasi.* This leads to misdiagnosis & poses a challenge in its diagnosis & treatment. The proposed study aimed at developing & evaluating candidate antigens for the diagnosis of VL. A total of 25 antigens were identified, recombinantly produced & evaluated using positive and negative VL controls. Three of these antigens Mitogen-activated protein kinase 3 (MAPK3), tryparedoxine & conserved hypothetical protein LDBPK gave sensitivities & specificities of over 80%. The study successfully evaluated their ability to diagnose VL using microbeads technology (over 80% sensitivity & specificity) & described their ability to differentiate between asymptomatic & symptomatic cases, which the rK39 kit is not able to differentiate. In addition, the study will attempt epitope mapping for the most promising antigen to identify peptides for developing a peptide diagnostic kit hence, increasing the sensitivity & specificity. Ultimately, the antigens will be evaluated in the field setting to access its suitability, acceptance, novelty & application as a point of care for VL.

1366

MEASURING THE IMPACT OF MASS TREATMENT WITH PRAZIQUANTEL ON SCHISTOSOMIASIS IN THREE BORDER COUNTIES IN LIBERIA: ASSESSING THE IMPACTS OF A DISRUPTED TREATMENT PATTERN

Anthony Kerkula Bettee[1], Karsor Kollie[2], Precious Z. Cooper Bettee[1]

[1]*Ministry of Health, Monrovia, Liberia,* [2]*Ministry of Health, Congo Town, P.0. Box 10-9009, 1000, Monrovia, Liberia*

Schistosomiasis (SCH) is among the neglected tropical diseases (NTDs) which remain a serious public health problem. The World Health Assembly resolution 54.19 urges all member states to regularly treat at least 75% of all school aged children who are at risk of morbidity from SCH through mass administration of preventive chemotherapy (PC). Liberia began PC for schistosomiasis in 2012, however due to the 2014-15 Ebola outbreak there was a large gap between the second and third treatment rounds. The second follow up survey was the first to take place since the resumption of treatment after Ebola, with the aim of determining changes in prevalence and intensity of SCH infection over time. An observational epidemiological survey was completed, utilizing a cross-sectional design whereby a new selection of children from the same age groups (6-11 years) were randomly sampled from sentinel schools in three high and moderate risk counties. *S Schistosoma mansoni* and *S. haematobium* infections both showed a significant decrease in prevalence between baseline (2012) and follow up 1 (2013): from 26.2% to 15.0% for S. *mansoni* and from 20.2% to 9.0% for S. *haematobium*. For follow up 2 in 2018, there was a slight increase in S. *haematobium* prevalence (from 9.0% to 9.8%), but an overall decrease from baseline, whereas for S. *mansoni* there was an increase in prevalence from baseline (from 26.2% to 35.7% at follow up 2), accompanied by an increase in the proportion of moderate and heavy intensity infections (from 0.2% at baseline to 7.0% at follow up 2). A third follow-up survey was completed in early 2020, however analysis of this data is ongoing and will form part of the presentation. It was interpreted that the spike of prevalence in follow up 2 may have been influenced by the cessation of MDA during the Ebola outbreak, low treatment coverage in particular areas, poor sanitation or other environmental factors. Our results emphasise the importance of routine and regular distribution of Praziquantel in line with WHO guidelines in order to ensure continued impact on SCH prevalence, and of working with programs to limit the impact on large-scale treatment programs during health systems shocks.

1367

LOCALISING EVIDENCE FOR DECISION-MAKING: A PARTICIPATORY AND AGENT-BASED MODELLING APPROACH TO INFORM AND ASSIST SCHISTOSOMIASIS CONTROL AND ELIMINATION

Cristin Alexis Fergus[1], Georgina Pearson[1], Liz Storer[1], Melissa Parker[2], Tim Allen[1]

[1]*London School of Economics and Political Science, London, United Kingdom,* [2]*London School of Hygiene & Tropical Medicine, London, United Kingdom*

The linear theories of change which ground many interventions do not account for the complex processes and systems in which they are implemented. This reductionist approach prioritizes statistical methods which do not accommodate the stochastic, non-linear, dynamic interactions between humans and their environment. The inclusion of practitioners in the process of evidence development and utilization of complex systems methods mitigates these issues and results in locally relevant, timely evidence for decision-making. The aim was to develop evidence for decision-making for schistosomiasis control and elimination in Uganda and Malawi. Workshops were conducted with practitioners from various levels within Ministries of Health and partner organizations to identify evidentiary needs for their decision-making processes and perceptions of disease transmission and control activities. Participatory systems mapping was used to identify factors directly and indirectly related to schistosomiasis transmission. The maps were synthesized to a master complex systems map, which served as the blueprint for a generalized spatial agent-based model and specific ABMs tailored to the evidence needs of decision-makers. The results following the first 3 workshops with 45 decision-makers highlighted a gap in available evidence for decision-makers to advocate for resources within the MoH and government budgets, intervention efficacy, and resource allocation within their purview. The scalable, adaptable, and data-inclusive characteristics of the AMBs made them well-suited to produce localized outputs which were responsive to the evidentiary needs of decision-makers from village to national levels and across country contexts. Used together, participatory and agent-based modelling present an opportunity to develop responsive and relevant evidence for practitioner decision-making. This process could be generalisable and transferable to diseases outside of schistosomiasis and locations outside of those in this study.

1368

EPIDEMIOLOGY OF SCHISTOSOMIASIS IN AN URBAN AREA OF THE CITY OF SALVADOR, BA, BRAZIL

Camila F. Chaves[1], Luciano K. Silva[1], Lúcio M. Barbosa[1], Mitermayer G. Reis[1], Ronald E. Blanton[2]

[1]*Oswaldo Cruz Foundation, BA, Salvador, Brazil,* [2]*Tulane School of Public Health and Tropical Medicine, New Orleans, LA, United States*

In Brazil, the recent demographic transition with the flow of people moving from the countryside to large cities has promoted the emergence of areas with precarious housing and sanitation conditions, and the re-urbanization of schistosomiasis. The objective of this study was to identify areas with focal transmission of schistosomiasis in Salvador-BA, Brazil. We conducted a cross-sectional study in 2019 in the Pirajá neighborhood, where the São Bartolomeu Park ecological reserve and the Cobre River basin are located. After free and informed consent, the participants answered a sociodemographic questionnaire and provided up to three stool samples on different days for parasitological examination using the Kato-Katz method. Additionally, four points were selected along the

Cobre River and one point in the neighborhood stream to determine the presence of *Biomphalaria* sp. and evaluate the fecal contamination of water collections using the Coliscan Easygel method. A total of 1,186 residents participated in the epidemiological and parasitological survey. The average age was 30.6 years (± 19.8 SD), most were female (55%), born in Salvador (85.4%) and with no history of travel to other regions in the last year (81 %). Based on the 2019 Brazilian Criteria, participants were grouped between classes B/C (56%) and D/E (44%) according to their socioeconomic conditions. Almost all households have access to piped water and sewage (99%). The prevalence of *Schistosoma mansoni* infection was 5.7% (95% CI 4.5% -7.2%), more than five times higher than the national average of 1.0% (95% CI 0.2% -1.8%) and twice as high as the estimate for the state of Bahia of 2.1% (95% CI 0% -4.4%), determined in the most recent National Survey of schistosomiasis mansoni and geohelminths (2010-15). Of the five water collection points, four presented fecal contamination and two the presence of *Biomphalaria* sp. In conclusion, the Pirajá neighborhood represents a focal point of schistosomiasis transmission in Salvador-BA. All positive participants were treated, and a new survey will be conducted in the same area.

1369

EVALUATION OF INTERVENTIONS AND RISK FACTORS CONTRIBUTING TO SCHISTOSOMIASIS PERSISTENCE IN SALVADOR, BRAZIL

Fernanda M. Cedraz[1], Luciano K. Silva[1], Mitermayer G. Reis[1], Ronald E. Blanton[2], Lucio M. Barbosa[1]

[1]*Oswaldo Cruz Foundation, BA, Salvador, Brazil,* [2]*Tulane School of Public Health and Tropical Medicine, New Orleans, LA, United States*

Rapid urbanization in Brazil is characterized by disordered migration from rural to urban areas, producing crowding communities with poor basic sanitation in cities such as Salvador, Bahia, Brazil. Saramandaia is a poor urban neighborhood reporting cases of schistosomiasis. The community is marked by the presence of streams and vegetable gardens, which was recently removed by the city government. This research aims to assess the effect of these interventions, treatment with praziquantel and risk factors in the urban environment on the persistence of schistosomiasis. In 2018, we conducted a sociodemographic and behavioral survey followed by examination using the Kato-Katz technique of up to three stool samples collected on different days. Those positive were treated with praziquantel and reexamined in four week. In 2019, another cross-sectional study was carried out on the same population following the same protocol. In 2018, 1799 participants were interviewed, and their average age was 31 ± 18 years, and 57% were female. Of these, 102 (5.7%) were *Schistosoma mansoni* egg-positive, with an average of 64 ± 110 eggs per gram of feces (epg). The prevalence was highest among the age groups 21 to 30 and 31 to 40 years (both 8%), but the highest intensity was in the age group 1 to 10 years (189 epg). Factors associated with schistosomiasis by multivariable analysis were male sex (OR = 2.5; p = <0.001), contact with surface water for work (OR = 1.7; p = 0.046) and contact with water at two points in the community with similar odds ratios (OR = 2.2; p = 0.005). Travel and birth outside of Salvador were not associated. On reexamination in 2019, 640 (35.6%) participated and 195 were included in the study, totalizing 835. The incidence was 2.2% (15/691), the rate of reinfection 2% (1/42), and the prevalence 2.8% (24/835) (p = 0.001). Infection in Saramandaia appears to occur in the neighborhood itself and it may be related to an occupational risk. The treatment of infected individuals has shown effectiveness in reducing prevalence. Structural interventions will be evaluated in future analysis.

1371

INTEGRATING WATER CONTACT BEHAVIOR, ENVIRONMENTAL HAZARD AND SOCIAL VULNERABILITY INTO ESTIMATES OF SCHISTOSOMIASIS OCCURRENCE IN NORTHERN SENEGAL

Andrea Lund[1], Susanne Sokolow[1], Isabel Jones[1], Andrew Chamberlin[1], Nicolas Jouanard[2], Simon Senghor[2], Assane Fall[2], Gilles Riveau[2], Giulio De Leo[1], David Lopez-Carr[3]

[1]*Stanford University, Stanford, CA, United States,* [2]*Centre de Recherche Biomedical Espoir pour la Sante, Saint-Louis, Senegal,* [3]*University of California, Santa Barbara, Santa Barbara, CA, United States*

Risk for schistosome infection is determined by three conditions: exposure, hazard and vulnerability. Exposure occurs through water contact, but without the hazard of schistosome cercariae in the water, infection does not occur. In turn, vulnerability, such as lack of access to piped water makes it difficult to reduce exposure in the presence of hazard. In this study, we aimed to understand how exposure, hazard and vulnerability contribute to the occurrence of schistosome infection. We use parasitology data from 1037 school-aged children in 16 villages in the lower basin of the Senegal River, survey data from the 530 households where those children reside and ecological data from 32 village water contact sites to (1) understand which conditions contribute to the best-fitting models of infection presence and intensity and (2) compare effect sizes of exposure, hazard and vulnerability indicators. We fit logistic and negative binomial regression models to infection presence and intensity data, respectively, adjusting for age and sex with random effects accounting for hierarchy in the data. We used Akaike Information Criterion to compare models. For both presence and intensity outcomes, model fit improved slightly with the incorporation of distance-weighted hazard and vulnerability indices. In the best-fitting presence models, effect sizes were larger for hazard (OR range 1.20 to 1.47) and vulnerability (OR range 1.48 to 2.04) compared to exposure indices (OR range 1.14 to 1.19). The effect sizes in the best-fitting intensity models were larger for exposure (IRR range 1.29 to 1.31) and vulnerability (IRR range 1.27 to 1.31) than hazard indices (IRR range 0.79 to 1.32). Our results suggest that it is important to jointly account for exposure, hazard and vulnerability as determinants of schistosome infection risk. Moreover, we find that hazard and vulnerability are the strongest determinants of infection presence while exposure and vulnerability are the strongest determinants of the accumulation of worms.

1373

OCCUPATIONAL RISK FOR *SCHISTOSOMA MANSONI* AND EVIDENCE FOR RAPID REINFECTION IN AGRICULTURAL WORKERS IN SALVADOR, BRAZIL

Pedro S. Mucillo[1], João R. Cruz[1], Luciano K. Silva[1], Adriano P. dos Santos[1], Mitermayer G. Reis[1], Ronald E. Blanton[2], Lúcio M. Barbosa[1]

[1]*Oswaldo Cruz Foundation, BA, Salvador, Brazil,* [2]*Tulane School of Public Health and Tropical Medicine, New Orleans, LA, United States*

Agricultural workers in Salvador, Bahia, Brazil have been described with a higher prevalence and intensities of *Schistosoma mansoni* infection than the general population. This study identified nearly all areas of commercial agriculture in the city and longitudinally monitored infected workers. Kato-Katz examinations were performed on up to 3 stools collected on different days at baseline, 1, 3, 4, 5 and 7 months followed by treatment at each period. Reinfection was assessed based on previous stool negativity and infrapopulation differentiation from previous samples. Population structure was assessed using 18 microsatellites. Across 8 neighborhoods, 18 gardens were identified. Crops were irrigated with surface water in 67%, and *Biomphalaria* sp. were present in 56%. All 36 workers participated. The majority were male (83.3%), with a mean age of 51 years. Previous schistosomiasis was reported by 36%. Across all sites, the prevalence was 25% with a mean egg burden of 367 ± 549 per gram of feces. Positives came from 2 sites. Component populations showed moderate differentiation (Dc = 0.101), thus gene flow was limited between sites

10 km apart. At 1 month, 2 workers were still positive due to taking no praziquantel or less than prescribed. One was persistent. Three months later, 1 worker who was negative after the first round of treatment became positive. At 4 months, all were negative on retreatment or first treatment (for 2 cases). At 7 months, 2 workers were again positive. Three not infected at baseline remained egg negative throughout. Differentiation between infrapopulations at baseline and 7 months (mean Di=0.26) indicates that reinfection was most likely, since there were periods of negative stools. A mere reduction in diversity of a persisting population is less likely. These studies indicate an intense transmission cycle with reinfection from local parasite pools. The role of these foci as a source for the rest of the community may be clearer as infrapopulations from the surrounding neighborhood are genotyped. There was no evidence of reduced efficacy, but reinfection and poor coverage explained most persistence.

1374

SCHISTOSOMA MANSONI ASSOCIATED MORBIDITY ALONGSIDE ANNUAL MASS DRUG ADMINISTRATION IN A HARD TO REACH DISTRICT OF MADAGASCAR

James M. Penney[1], Stephen A. Spencer[2], Cortland Linder[3], Hannah Russell[3], Kate Hyde[3], Jemima Henstridge-Blows[3], Isla Young[3], Russell J. Stothard[4], Amaya L. Bustinduy[5], Alain Rahetilahy[6]

[1]*Greater Glasgow and Clyde NHS Trust, Glasgow, United Kingdom,* [2]*North Bristol NHS Trust, Bristol, United Kingdom,* [3]*University of Manchester, Manchester, United Kingdom,* [4]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [5]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [6]*Ministère de la Santé Publique, Antananarivo, Madagascar*

Schistosomiasis has a huge burden of morbidity in Madagascar. Madagascar Medical Expeditions (Madex) is a voluntary organisation set up by students from the University of Manchester (UK) to tackle schistosomiasis in the hard to reach Marolambo district, in collaboration with the Madagascar Ministry of Health. In 2015, Madex identified a 94% prevalence of *Schistosoma mansoni* in school-aged children (SAC; aged 5-14 years) in the district. Between 2016 and 2019, disease related morbidity in SAC was measured alongside annual mass drug administration (MDA) of Praziquantel. Repeated cross-sectional surveys were carried out in six villages in the Marolambo district. Various parameters of morbidity were measured: haemoglobin levels to assess for anaemia, 20-metre shuttle run to assess cardiovascular fitness by calculation of VO2 max and anthropometrics to assess for presence of stunting and/or wasting. MDA was delivered to the population annually. There was no significant improvement in morbidity from 2016 to 2019. Percentage of children with anaemia went from 58% (173/298) to 60% (240/400), ranging from 58% (173/298) to 76% (228/300). Average VO2 max went from 46.1ml/min/kg to 47.3ml/min/kg, ranging from 46.1ml/min/kg to 47.3ml/min/kg. Proportion of stunted children went from 55% (166/300) to 52% (175/340), ranging from 37% (146/400) to 55% (166/300). Proportion of wasted children went from 8% (24/300) to 9% (31/344), ranging from 8% (24/300) to 23% (92/400). Four years of annual MDA has not led to significant improvements in schistosomiasis associated morbidity in this high disease-burden population. Many more years of consistent MDA are needed to see significant improvements to the chronic morbidity brought about by intestinal schistosomiasis in this region.

1375

SENSITIVE DIAGNOSTIC TOOLS AND TARGETED DRUG ADMINISTRATION STRATEGIES ARE NEEDED TO ELIMINATE SCHISTOSOMIASIS

Govert J. van Dam[1], Abena S. Amoah[1], Pytsje T. Hoekstra[1], Miriam Casacuberta Partal[1], Luc E. Coffeng[2], Paul L. Corstjens[3], Beatrice Greco[4], Lisette van Lieshout[1], Mark D. Lim[5], Christine F. Markwalter[6], Maurice R. Odiere[7], Jutta Reinhard-Rupp[4], Russell Stothard[8], Louis-Albert Tchuem Tchuenté[9], Sake J. de Vlas[2]

[1]*LUMC - Dept. of Parasitology, Leiden, Netherlands,* [2]*Erasmus University Medical Center - Dept. Public Health, Rotterdam, Netherlands,* [3]*LUMC - Dept. of Cell and Chemical Biology, Leiden, Netherlands,* [4]*Merck Global Health Institute, Eysins, Switzerland,* [5]*The Bill & Melinda Gates Foundation, Seattle, WA, United States,* [6]*Vanderbilt University - Dept. of Chemistry, Nashville, TN, United States,* [7]*Kenya Medical Research Institute, Kisumu, Kenya,* [8]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [9]*University of Yaoundé I - Lab. of Parasitology and Ecology, Yaoundé, Cameroon*

Although preventive chemotherapy has been instrumental in reducing schistosomiasis incidence worldwide, serious challenges remain. These problems include the omission of certain groups from campaigns of mass drug administration, the existence of persistent disease hotspots, and the risk of recrudescent infections. Central to these challenges is the fact that the diagnostic tools currently used to establish the burden of infection are not sensitive enough, especially in low-endemic settings, which results in underestimation of the true prevalence of active *Schistosoma* infections. This central issue necessitates that the current schistosomiasis control strategies recommended by WHO are re-evaluated and, possibly, adapted. More targeted interventions and novel approaches have been used to estimate the prevalence of schistosomiasis, such as establishing infection burden by use of precision mapping, which provides high resolution spatial information that delineates variations in prevalence within a defined geographical area. Such information is instrumental in guiding targeted intervention campaigns. However, the need for highly accurate diagnostic tools in such strategies is a crucial factor that is often neglected. The availability of highly sensitive diagnostic tests also opens up the possibility of applying strategies of sample pooling to reduce the cost of control programmes. To interrupt the transmission of, and eventually eliminate, schistosomiasis, better local targeting of preventive chemotherapy, in combination with highly sensitive diagnostic tools, is crucial.

1376

SCHISTOSOMIASIS AMONG VILLAGES SURROUNDING LAKE NYASA, SOUTHERN TANZANIA: PREVALENCE, INTENSITY OF INFECTION AND GEOGRAPHICAL DISTRIBUTION AMONG PRE-SCHOOL & SCHOOL AGED CHILDREN

Humphrey D Mazigo[1], Cecilia Uisso[2], Paul Kazyoba[2], Andreas Nshala[2], Upendo Mwingira[3]

[1]*Department of Medical Parasitology, School of Medicine, Catholic University of Health and Allied Sciences, Mwanza, United Republic of Tanzania,* [2]*National Institute of Medical Research, Dar es Salaam, United Republic of Tanzania,* [3]*RTI, Maryland, WA, United States*

Planning and implementation of schistosomiasis control activities requires an understanding of the prevalence, intensity of infection and geographical distribution of the disease in different epidemiological settings. Although, Tanzania is known to be highly endemic to schistosomiasis, there is paucity of data on the geographical distribution of schistosomiasis in potential large water bodies in the country. Thus, the present study was conducted to determine the prevalence, infection intensities and geographical distribution of schistosomiasis along villages located on the shoreline of Lake Nyasa, southern Tanzania. A cross-sectional study was conducted among 1,560 children aged 1-13 years old living in villages located along the shoreline of Lake Nyasa. A single urine and stool sample was obtained from each participating child and screened for *S. mansoni* using Kato Katz (KK) technique to detect eggs

and using point-of-care circulating Cathodic Antigen (POC-CCA) test to detect antigen in urine. Urine filtration technique was used to screen for *S. haematobium* eggs in urine samples. Villages/primary school were mapped using geographical information system and prevalence map was generated using ArcView GIS software. The overall prevalence of *S. mansoni* based on KK technique and POC-CCA test was 15.1% (95%CI:13.4-16.9) and 21.8% (95%CI:18.5-25.3) respectively. The prevalence *S. haematobium* was 0.83% (95%CI:0.5-1.4) and that of haematuria was 0.9%. The arithmetic mean egg intensities for *S. haematobium* and *S. mansoni* were 18.5 mean eggs/10mls(95%CI:5.9-57.6) of urine and 34.7mean epg (95%CI:27.7-41.7) respectively. Villages located on the southern end of the lake had significantly high prevalence of *S. mansoni* than those located on the northern part (χ^2=178.7838, *P*=0.001). Cases of *S. haematobium* were detected only in three villages. Both *S. mansoni* and *S. haematobium* infections occurs in villages located along the shoreline of Lake Nyasa at varying prevalence. These finding provide insights that can provide guidance in planning and implementation of MDA approach to control schistosomiasis.

1377

IS KNOWLEDGE POWER OR A PREDICTOR? ASSESSING PARENTAL EDUCATION ASSOCIATIONS WITH *SCHISTOSOMA HAEMATOBIUM* INFECTION IN KANO STATE, NIGERIA

Angela Udongwo

Rollins School of Public Health, Emory University, Decatur, GA, United States

There are about 250 million people infected with schistosomiasis worldwide and 300,000 people dying annually. Urogenital schistosomiasis (*Schistosoma haematobium*) infects school-aged children at an alarming rate and there is a need to evaluate community-based factors' associations with the infection. This study aims to compare infection across levels of parental education in Hausa communities of Kano State, Nigeria. The data is a part of the cross-sectional study 'Kano Schistosomiasis in SAC 2019', taken from participants residing in five local government areas in Kano State. Urine samples were collected and the presence of *S .haematobium* eggs were examined utilizing urine filtration. Tests for association were conducted and a multivariate logistic regression model was used to predict the presence of urogenital schistosomiasis. Of the 272 participants, the overall prevalence of urogenital schistosomiasis was 36.9%. The mean age of the study sample was 11 years (4.08 SD). There were 218 (81.34%) males and 50 (18.66) females. The highest caseload was amongst the 12-18-year-old group (45.6%). Out of the infected study sample, mid-range (secondary and technical) parental education had the highest rate of infection (45.9%) compared to low-range (no education-primary level) education and high-range (professional and university) education (35.6% and 18.4% respectively). The multivariate logistic regression analysis revealed that mid-range education (aOR = 1.293, 95% CI: 0.610-2.740), and low-range education (aOR= 2.218, 95% CI: 0.962-5.114) did not have a statistically significant difference across ranges. Questionnaire data revealed 64.3% (173) reported engaging in activities with unprotected water sources, 40.9% (110) did not have knowledge on transmission, and 14.5% (39) reported having a past "worm" disease. The study shows a high caseload of infected children in Hausa communities within Kano State, Nigeria. Although there was not a significant association found between parental education and infection, it is evident that Hausa communities require an integrated control approach and an increased awareness of the disease.

1378

A PROSPECTIVE COHORT STUDY OF CHILD MOUTHING OF FECES AND FOMITES IN URBAN DHAKA, BANGLADESH (CHOBI7 PROGRAM)

Tahmina Parvin[1], Ismat Minhaj Uddin[1], Md. Sazzadul Islam Bhuyian[1], Ronald Saxton[2], Fatema Zohura[1], Marzia Sultana[1], Fatema-Tuz Johura[1], Shirajum Monira[1], M Tasdik Hasan[3], Nowshin Papri[1], Md. Ahshanul Haque[1], Shwapon Kumar Biswas[1], Shwapon Kumar Biswas[4], David A. Sack[2], Jamie Perin[2], Munirul Alam[1], **Christine Marie George**[2]

[1]*International Center for Diarrheal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh,* [2]*Johns Hopkins University, Baltimore, MD, United States,* [3]*University of Liverpool, London, United Kingdom,* [4]*Rangpur Medical College and Hospital, GOB, Dhaka, Bangladesh*

To characterize childhood mouthing and handling behaviors and to assess the association between hand-to-object and object-to-mouth contacts and diarrhea prevalence in young children in urban Dhaka, Bangladesh. A prospective cohort study was conducted among 494 children under 5 years of age in Dhaka, Bangladesh. This study was nested within the randomized controlled trial of the Cholera-Hospital-Based-Intervention-for-7-Days (CHoBI7) mobile health (mHealth) program. The CHoBI7 mobile health program focuses on promoting handwashing with soap and water treatment to diarrhea patients and their household members through mobile messages and in-person visits..Mouthing and handling of feces and fomites among young children was measured by five-hour structured observation and caregiver reports. Diarrhea surveillance data was collected monthly for 12 months. Fifty-five percent of caregivers reported that their child put a visibly dirty fomite (object or soil) in their mouth in the past week. Fifty percent of children had caregiver reports of mouthing visibly dirty objects, 26% had reports of mouthing dirt, and 2% had reports of mouthing feces. Forty-five percent of children were observed mouthing a visibly dirty fomite during structured observation. Forty percent of children were observed mouthing an object, 10% were observed mouthing soil, and one child (0.2%) was observed mouthing feces. Mouthing of visibly dirty fomites was highest for children 12-18 months of age with 69% of these children having caregiver reports and 54% having observed events. Children with caregiver reports of mouthing feces had a significantly higher odds of diarrhea over the subsequent month (Odds Ratio: 4.54; 95% Confidence Interval: 1.06, 19.48). These findings demonstrate that mouthing of contaminated fomites among young children is frequent in urban environments in Bangladesh, and that mouthing feces is associated with a significantly higher odds of diarrhea. Interventions are urgently needed to protect young children from fecal pathogens in their play spaces.

1379

CHILD MOUTHING OF SOIL AND ANIMALS PRESENCE IN CHILD SLEEPING SPACES ARE ASSOCIATED WITH GROWTH FALTERING AMONG YOUNG CHILDREN IN URBAN DHAKA, BANGLADESH (CHOBI7 PROGRAM)

Shirajum Monira[1], Md. Sazzadul Islam Bhuyian[1], Tahmina Parvin[1], Ismat Minhaj Uddin[1], Fatema Zohura[1], M Tasdik Hasan[1], Shwapon Kumar Biswas[1], Khaled Hasan[2], Jahed Masud[1], Mahamud-ur Rashid[1], Zillur Rahman[1], Nowshin Papri[1], Raisa Rafique[1], Aminul Islam[1], Indrajeet Barman[1], Fatema Tuz Jubyda[1], Fatema-Tuz Johura[1], Marzia Sultana[1], Kazi Istiaque Sanin[1], David A. Sack[2], Jamie Perin[2], Munirul Alam[1], **Christine Marie George**[2]

[1]*International Center for Diarrheal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh,* [2]*Johns Hopkins University, Baltimore, MD, United States*

The objective of the study was to investigate potential risk factors for growth faltering among children under than 5 years of age. We conducted a prospective cohort of 594 children under 5 years from diarrhea patient households in urban Dhaka, Bangladesh. Anthropometric measurements were assessed at baseline and at a 12-month follow-up. Caregivers of

young children were administered a monthly questionnaire on household sociodemographic characteristics and hygiene practices. Children with caregiver reports of mouthing of soil at the majority of household visits had a significant reduction in their height for age z-scores (HAZ) from baseline to the 12-month follow-up (ΔHAZ -0.28 (95% Confidence Interval (CI): -0.51, -0.05)). A significant reduction in HAZ was also observed for children in households with animals in their sleeping space (ΔHAZ -0.35 (95% CI: -0.68, -0.03)). These findings provide further evidence to support the hypothesis that child mouthing of soil and the presence of animals in the child's sleeping space are potential risk factors for growth faltering in young children. Interventions are needed to provide clean play and sleeping spaces for young children to reduce exposure to fecal pathogens through child mouthing.

1380

SUSTAINED WASH BEHAVIOR CHANGE AMONG HOUSEHOLD MEMBERS OF DIARRHEA PATIENTS (CHOBI7 PROGRAM): LESSONS LEARNED FROM DOERS AND NON-DOERS

Elizabeth D. Thomas[1], Fatema Zohura[2], Md. Sohel Rana[1], Alana Teman[1], Tahmina Parvin[2], Jahed Masud[2], Md. Sazzadul Islam Bhuyian[2], Sanya Tahmina[3], Farzana Munmun[4], Md. Abul Hashem Khan[4], David A. Sack[1], Elli Leontsini[1], Peter J. Winch[1], Munirul Alam[2], **Christine Marie George**[1]

[1]*Johns Hopkins University, Baltimore, MD, United States,* [2]*International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh,* [3]*Ministry of Health and Family Welfare, Dhaka, Bangladesh,* [4]*Directorate General of Health Services, Dhaka, Bangladesh*

Sustained behavior change is important for the success of WASH interventions. The Cholera Hospital-based Intervention for 7 Days is a hospital-initiated water treatment and handwashing with soap intervention to reduce transmission of diarrhea between patients and their household members. The intervention includes a pictorial module on diarrhea transmission, handwashing station, soapy water bottle, drinking water container, and chlorine tablets for water treatment. We conducted interviews with households 6-28 months post-enrollment, purposefully selecting households no longer using enabling technology (non-doers) or households still using the drinking water container, handwashing station, or soapy water bottle (doers). The objectives were to explore barriers and facilitators to recommended behaviors and to inform modifications to the intervention ahead of larger-scale implementation. Households using the handwashing station disliked their previous handwashing system (e.g. communal pump). Barriers to using the handwashing station included young children spilling water, hardware breakage, and limited household space for the technology. Some households stopped using their soapy water bottle because children would spill the contents. A few households solved this problem by tying the soapy water bottle out of reach of children. Reasons for not using the water container included having an alternate system (e.g. water filter), limited space, and hardware breakage. Other reasons for no longer using the hardware included gifting items to family members or shifting homes and leaving hardware behind. Based on the findings from doers and non-doers, we provided households with additional behavior change communication, including support for repairing hardware and recommending to elevate the soapy water bottle on a string to avoid spillage. WASH behavior change interventions should provide households with support during disrupting events (e.g. moving), develop child-friendly components, and provide guidance on repair or purchase of technology in case of breakage.

1381

IMPLEMENTING INTEGRATED WASH, NUTRITION, AND EARLY CHILDHOOD DEVELOPMENT INTERVENTIONS - COMMUNITY HEALTH WORKERS' EXPERIENCES FROM BANGLADESH

Tania Jahir[1], Mahbubur Rahman[1], Elli Leontsini[2], Farzana Yeasmin[1], Stephen P. Luby[3], Peter J. Winch[2]

[1]*icddr,b, Dhaka, Bangladesh,* [2]*John Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [3]*Stanford University, Stanford, CA, United States*

There are increasing calls to integrate water, sanitation and hygiene (WASH) with other maternal and child health interventions. In Bangladesh, we piloted an intervention package integrating WASH with prevention of lead and arsenic exposure, child feeding and early childhood stimulation in 31 (16 intervention and 15 control) villages. Community Health Workers (CHW) delivered the intervention fortnightly to pregnant and mothers of baby less than 2 years of age, through group meetings and home visits. We assessed the feasibility, acceptability, challenges, and opportunities for the CHWs of intervention delivery through 6 focus groups and 4 in-depth interviews. CHWs and their supervisors reported the integration as an opportunity, enabling them to link handwashing to child feeding, collecting arsenic-free water for a child's meal, and storing the food in a lead-free container. They also reported child stimulation as an opportunity by advising caregivers to teach a child about handwashing while talking and interacting with the child through age specific homemade toys and introducing about various objects and surrounding environment through picture books. Feasibility increased with group sessions compared with individual home visits. Challenges included the higher investment needed for WASH compared to nutrition and stimulation interventions, the long duration of CHW training, conducting group sessions in the presence of noisy children, and participants' late arrivals. Integration was feasible for implementing CHWs in this setting. Challenges can be overcome by revising CHW training and caregivers' group session duration, logistics, content and accompanying materials. Respect, support and acceptance from both community and supervisors worked as motivators for CHWs, so these can be carried on for future.

1382

MEASURING THE WATER QUANTITY FOR PERSONAL AND DOMESTIC HYGIENE AND DETERMINANTS OF WATER USE IN LOW-INCOME URBAN COMMUNITY

Rebeca Sultana[1], Nadia Ali Rimi[1], Nazmun Nahar[2], Syeda Tasnuva Swarna[1], Shifat Khan[1], Humayun Kabir[1], Khaled Saifullah[1], Peter Kjær Mackie Jensen[3]

[1]*icddr,b, Dhaka, Bangladesh,* [2]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [3]*Copenhagen Center for Disaster Research, Department of Public Health,, University of Copenhagen, Denmark*

Quantity of water for hygiene plays an important role in diarrheal disease transmission. Much of the evidences rely on water access as proxy for water quantity and there is a paucity of recent research on direct water quantity measurement for hygiene which can provide an important insight on water and disease related research. The study aimed to measure the water usage for personal and domestic hygiene and to explore the reasons for variation of water usage by month and by availability of water of a low-income urban community in Bangladesh. The team conducted a day long (12 to 14 hours a day) bimonthly observation for one year in each of 12 purposively selected households to record volume of water use per activity of the household members. They also conducted 28 in-depth interviews with female and male residents of 24 households and explored the reasons for changes in water use practices for personal and domestic hygiene. All the study households' members were Muslim. The median water use per person per day was 75 liters including for personal hygiene 39 liters. Those who practice religious prayer regularly used more water than those who do not practice prayer regularly (median 49 vs. 36 liters) per day. Participants reported maintaining religious rule and rituals

properly requires more water than other purpose. The rationale for using more water Participants used more water per person per day for personal hygiene in hot month of June than cold month of January (median 40 vs 30 liters). When water was available for 24 hours participants used more water for personal and domestic hygiene than when it was available for <24 hours (median 79 vs 65 liters per person per day). The amount of water use varied among participations due to weather, availability and practicing religious rituals. Motivation and rationale for water use were embedded in individuals' behavioral factors including maintaining religious rule, water availability and weather determinants. Future WASH related targets and indicators should incorporate behavioral factors that determine water quantity for personal and domestic hygiene.

1383

COMPARATIVE ASSESSMENT OF FECAL CONTAMINATION IN 'IMPROVED' PIPED-TO-PLOT COMMUNAL SOURCE AND POINT-OF-DRINKING WATER

Jannatul Ferdous[1], Rebeca Sultana[2], Ridwan Rashid[1], Anowara Begum[1], Peter Kjær Mackie Jensen[3]

[1]*University of Dhaka, Dhaka, Bangladesh,* [2]*icddr,b, Dhaka, Bangladesh,* [3]*University of Copenhagen, Copenhagen, Denmark*

The aim of this study was to compare the water quality of piped-to-plot communal source with point-of-drinking water in households of a low-income urban area in Bangladesh. Drinking water samples were taken directly from households i.e. point-of-drinking (n=2,514) water and from their linked communal source water (n=1,926) for basic water quality analysis for *Escherichia coli* (*E. coli*) using membrane filtration culture method. Paired data of connected communal source and point-of-drinking water collected on the same day showed that the level of fecal contamination increased from communal source to point-of-drinking water of the linked households in 51% (626/1,236) of samples. A total of 38% of point-of-drinking water samples, communal source had comparatively cleaner sources (26% had *E. coli:* 0 CFU/100 mL and 12% had *E. coli*: 1-10 CFU/100 mL) and this level had subsequently increased in the point-of-drinking water samples. Results also showed that 78% of (260/333) treated and 76% (1,662/2,175) of non-treated point-of-drinking water were found to be contaminated with *E. coli.* Comparison between bottle vs other wide mouth vessels (i.e. glasses, mugs, jugs) showed significant lower odds (p=0.007, OR=0.68, [0.51-0.90]) of fecal contamination for >100 *E. coli*/100 mL compared to other drinking vessels. Our study reveals that recontamination and post contamination at point-of-drinking plays significant role for water contamination at domestic domain. To reduce domestic transmission of fecal-oral pathogens, hygiene education efforts should target to improve kitchen hygiene practices including safe handling of drinking water after treatment and promotion of narrow mouth drinking vessels can be encouraged.

1384

LOCAL PERCEPTIONS, CULTURAL BELIEFS, PRACTICES AND CHANGING PERSPECTIVES OF HANDLING INFANT FECES: A CASE STUDY IN A RURAL GEITA DISTRICT, NORTH-WESTERN TANZANIA

Joy J. Chebet[1], Aminata Kilungo[1], Halimatou Alaofè[1], Hamisi Malebo[2], Shaaban Katani[2], Mark Nichter[1]

[1]*Univeristy of Arizona, Tucson, AZ, United States,* [2]*Tanzania National Institute for Medical Research, Dar es Salaam, United Republic of Tanzania*

We report on the management of infant feces in a rural village in Geita region, Tanzania. Findings discussed here emerged incidentally from a qualitative study aimed at investigating vulnerability and resilience to health challenges in rural settings. Data was gathered through semi-structured Focus Group Discussions (FDGs) with women (n=4; 32 participants), men (n=2, 16 participants), and community leaders (n=1; 8 participants). All FDGs were audio recorded, transcribed verbatim and thematically analyzed using Atlas.ti. Respondents reported feces of a child under the age of six months were considered pure compared to that of older children. Infant feces were seen as transitioning to harmful at the point when the child began to eat solid food, resulting in their stool visually changing in appearance. Caregivers reportedly used soft implements to handle infant feces due to the belief that tools with hard surfaces would physically harm the child. Infant feces were disposed in environments around the house due to the belief that disposal in latrines would prevent developmental milestones and result in other perceived negative health outcomes for the child. Changing views expressed by participants suggest a window of opportunity to implement evidence-based, and culturally-relevant interventions to encourage the safe disposal of infant feces.

1385

MEASURING EFFECT OF A MENSTRUAL HYGIENE MANAGEMENT INTERVENTION THROUGH SCHOOL PERFORMANCE OF GIRLS IN BANGLADESH

Farhana Sultana[1], Shirina Aktar[1], Shifat Khan[1], Farhana Akand[1], Md. Nuruzzaman[1], Supta Sarker[1], Md. Mahbubur Rahman[1], Leanne Unicomb[1], Peter J. Winch[2], Stephen P. Luby[3], Fahmida Tofail[1]

[1]*icddr,b, Dhaka, Bangladesh,* [2]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [3]*Stanford University, Stanford, CA, United States*

Around one-third of Bangladeshi girls perceive that a lack of menstrual hygiene management (MHM) facilities in schools contributes to poor school performance. To determine the impact of MHM intervention, we need reliable measures of school performance. In this pilot study, we validated a school performance test, based on Wide Range Achievement Test (WRAT). The revised WRAT measured 3 domains of literacy and numeracy: reading, spelling and math computation, among 200 randomly selected girls before and after a 6-month intervention in July 2017 to April 2018. We performed correlational analyses, independent sample t-test, chi-squared test for validity, internal consistency and reliability (test-retest). The intervention included: Products-cloth pads, underwear, plastic bag (to take the stained cloth pad home to wash/dry) and menstrual calendars, Education curriculum-flipcharts, comic books and training teachers to deliver MHM sessions, and Maintenance-chute disposal for disposable pads and gender committees to promote intervention activities. The mean age of the girls was 13.3 (SD1.6) years. All girls understood the tasks and followed instructions readily, suggests excellent face validity. The internal consistency of subscales of similar underlying constructs gave r value of between 0.65-0.70, test-retest (after 7 days) and inter-rater reliabilities had r>0.80. Adjusted scores increased after the intervention in 3 domains (mean difference range 0.8-1.07). Subscale scores correlated highly (r >0.74) between two time points of measurement in both urban and rural schools, indicating good reliability of the measure. Meaningful positive correlation of scores for one or more domains of the test with family house constructions, maternal education and parental occupation suggests contribution of socio-demographic factors to girls' school performance. This was a valid and reliable test to assess MHM intervention effect on school performance. An RCT to determine MHM intervention impact should include school performance outcome measures.

1386

HEAVY METAL RESIDUAL ANALYSIS OF TOMATO IN THE HOHOE MUNCIPAL MARKET, GHANA

Forgive A. Norvivor, Nicholas O. Opoku

University of Health and Allied Sciences, Ho, Ghana

In recent years, there has been an increasing ecological and global public health concern associated with environmental contamination by heavy metals. The rampant use of these chemicals, under the adage, "*if little is good, a lot more will be better*" has played havoc with human and other life forms. In Ghana, some farmers engage in indiscriminate application of agrochemicals many times during the cropping season, with illiteracy and negligence being a major reason for misapplications. Study to investigate

the concentration of heavy metals in tomatoes sampled from the Hohoe municipality market is imperative. Atomic Absorption spectrophotometry (AAS) method. Results from this on-going research shows quite interesting results, whereby Pb &gt Cd &gt Hg &gt As &gt Cr was a major concentration trend in all four different tomato sale whole sites in the Hohoe market.The outcome of this study will be a baseline data on the quality of crop being consumed with respect to heavy metals and it would also be a basis for further studies into investigating the quality of other staple foods/crops.

1387

EXPOSURE TO FECAL PATHOGENS AMONG INFANTS IN URBAN ECUADOR: DEVELOPMENT OF A STRUCTURED OBSERVATION INSTRUMENT

Andrea Sosa-Moreno[1], Gwenyth O. Lee[1], Amanda Van Engen[1], Kelly Sun[1], Jessica Uruchuma[1], Laura Kwong[2], Elizabeth Ludwig-Borycz[1], Bethany Caruso[3], William Cevallos[4], Karen Levy[3], Joseph N. Eisenberg[1]

[1]*University of Michigan, Ann Arbor, MI, United States,* [2]*Stanford University, Stanford, CA, United States,* [3]*Emory University, Atlanta, GA, United States,* [4]*Instituto de Biomedicina, Carrera de Medicina, Universidad Central del Ecuador, Ecuador*

Although childhood diarrhea is one of the leading causes of morbidity and mortality in children under five, we know little about the relative contributions of varying exposure pathways to infection. To characterize potential sources of exposure, structured observations are used to complement to survey data and environmental sampling. New software-based tools also allow observation data to be quantitatively summarized. However, to ensure that the most relevant behaviors are fully characterized, appropriate tailoring to the local context is necessary. Here, we describe the systematic development and implementation of a structured observation tool using LiveTrak, a software to track the initiation and duration of events that may create risks of infection. This tool is intended to (1) characterize hygiene behaviors during key activities (hand washing, diaper changing, and feeding) and, (2) to estimate the intensity of oral contact events among infants in coastal Ecuador. To develop this tool, we identified locally relevant themes from a pilot study that trialed a series of structured prompts and collected extensive, unstructured fieldworker notes. The structured observation was then translated into LiveTrak and implemented in an ongoing cohort study of childhood diarrhea. During piloting, hygiene behaviors and the frequency of contact events (events per hour) were observed among 45 infants for a total of 166 hours. As of April 1st, 2020, the final observation had been completed among 17 cohort infants for a total of 45.5 hours. The frequency, ordering, and duration of key hygiene behaviors, and 'contact episodes', defined as periods of frequent touching or mouthing of other humans, animals, surfaces and play objects, was ascertained. This systematic and reproducible process of instrument development can be utilized to develop context-specific, but comparable, estimates of locally relevant disease transmission processes. In our ongoing cohort study, information from the structured observation will be combined with questionnaire data, spot checks, and environmental microbiology to triangulate estimates of environmental exposure.

1388

BURDEN OF CHOLERA IN NORTHWESTERN NIGERIA. AN ANALYSIS OF TREND OF CASES BETWEEN 2014-2019 IN KANO STATE, NIGERIA

Usman L. Shehu

AFENET/Nigeria Field Epidemiology and Laboratory Training Program, Abuja, Nigeria

Cholera remains a global threat and is one of the key indicators of social development. It is a disease of public health importance and remains a challenge to countries where access to safe drinking water and adequate sanitation cannot be guaranteed. The global burden of cholera is largely unknown because the majority of cases are not reported and can be attributed to limited capacity of epidemiological surveillance and laboratories. An update of the burden of cholera is needed to assist public health practitioners and policy-makers in cholera control efforts. This study evaluated the surveillance data from all suspected and confirmed cases reported to the most populous state of Nigeria for the last 6 years. A case was defined as any person above the age of 5 years with acute watery diarrhoea of rapid onset. Line listing of suspected cases and deaths were collected by the Integrated Disease Surveillance and Response (IDSR) team from January 2014 to Dec 2019. During January 2014 to December 2019, a total of 12,584 cholera cases were reported from the 44 Local government areas of Kano to the IDSR with the highest cases (10,307) in 2014 and lowest (0) in 2016. Out of the total reported cases, only 118 (0.9%) cases were laboratory confirmed positive for Vibrio cholera serotype 01. In 2017, a total of 358 suspected and 8 confirmed cases were reported while in 2018, a total of 288 suspected and 1 confirmed case was reported. In 2019, a total of 89 cases were reported with only 9 confirmed. Majority of the reported cases were from the rural LGAs of Gaya (5.2%) and Dawakin Kudu (5.2%) which lack basic amenities while the least (0.001%) is from Kunci LGA. The burden of cholera is high but there is decreasing trend of cases across the years. There is need for increased laboratory confirmation of cases, cholera vaccination and provision of adequate water and environmental sanitation to the rural populations at risk in order to achieve control.

1389

ACCESS TO ALCOHOL-BASED HAND RUB AND HAND HYGIENE ADHERENCE AMONG HEALTHCARE PROFESSIONALS IN KABAROLE DISTRICT, UGANDA, 2018-2019

Matthew Lozier[1], Maureen Kesande[2], Sunkyung Kim[1], Patricia Akers[1], Olive Tumuhairwe[3], Martin Watsisi[4], Winifred Omuut[2], Margaret Person[1], Mohammed Lamorde[2], Jennifer Murphy[1], Robert Quick[1], David Berendes[1]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Infectious Diseases Institute, Kampala, Uganda,* [3]*Kabarole District Health Office, Ministry of Health, Fort Portal, Uganda,* [4]*International Water and Sanitation Centre – WASH, Fort Portal, Uganda*

Alcohol-based hand rub (ABHR) can improve healthcare professional (HCP) hand hygiene adherence (HHA) in healthcare facilities (HCF) in high-income countries, protecting HCPs from acquiring or spreading infectious diseases such as COVID-19 and Ebola. However, commercial ABHR is expensive and often unavailable in low-income settings. To assess associations between ABHR availability and HHA in Kabarole District, Uganda ("Kabarole"), we distributed locally produced ABHR in February 2019 to HCF and anonymously recorded HHA of HCPs before and after patient contact. HHA was observed at baseline (August 2018, pre-ABHR distribution), midpoint (June 2019), and endpoint (November 2019). HHA was defined as handwashing with soap and water or ABHR use. HHA was recorded from 17 HCF (209 patient contacts, 43 HCPs) at baseline, 30 HCF (306 contacts, 69 HCPs) at midpoint, and 30 HCF (308 contacts, 69 HCPs) at endpoint. Across all timepoints, HCPs included nurses (34%), midwives (23%), clinical officers (13%), and lab technicians (30%). HHA *before* patient contact was 0% at baseline, 23% at midpoint (18% ABHR, 5% handwashing), and 9% at endpoint (9% ABHR, 0% handwashing). HHA *after* patient contact was 15% at baseline (3% ABHR, 12% handwashing), 66% at midpoint (58% ABHR, 8% handwashing), and 41% at endpoint (38% ABHR, 3% handwashing). In multivariable logistic regression using 15 HCF that participated at all timepoints, odds of HHA *after* patient contact at midpoint and endpoint were 16.9 (95% CI=4.5–63.3, p<0.001) and 5.2 (95% CI=1.4–18.6, p=0.012) times higher than at baseline, respectively. The odds of HHA at endpoint was 0.3 times lower than at midpoint (95% CI: 0.1–0.7, p=0.002). Modeling controlled for HCF and HCP type and invasive vs non-invasive contact. HHA in Kabarole HCF increased 4 months after ABHR distribution and decreased 5 months later but remained higher than at baseline. Increased HHC at midpoint may reflect efforts to improve HHA in response to identification of imported

Ebola cases near Kabarole 2 weeks before midpoint data collection. ABHR availability increased HHA, but additional interventions to increase HHA further may be warranted.

1390

PERSISTENCE OF *SALMONELLA* TYPHI VIABILITY AND DNA IN SEWAGE

Renuka Kapoor, Christine L. Moe

Center for Global Safe Water, Sanitation, and Hygiene, Emory University, Atlanta, GA, United States

Typhoid fever caused by *Salmonella enterica* serovar Typhi (ST) remains a major public health problem in low- and middle- income countries. Typhoid incidence remains poorly characterized due to lack of standardized clinical surveillance and inefficient diagnostics. Sewage-based environmental surveillance can be used to complement clinical surveillance, given that ST may be shed in the feces during and following both clinical and subclinical infection. Understanding ST survival and the persistence of ST DNA in sewage is important for interpreting environmental surveillance data. Here, we report the findings from our study of ST persistence in sewage. Propidium monoazide- quantitative real time PCR (PMA-qRTPCR) was developed to determine the viability of ST bacterial cells for monitoring persistence in sewage. Mixtures with varying concentrations (10^8, 10^5 and 10^3 cells) of viable and non-viable (heat-killed) ST cells were treated with different concentrations (10 µM-200 µM) of PMA, followed by DNA extraction and qRTPCR using ST2RK primers targeting the *stgA* gene. Optimized PMA treatment conditions were used to study persistence of ST in sewage at different temperatures (37ºC, 25ºC, and 4ºC) over a period of 30 days. PMA-qRTPCR showed a gradual decline in signal (increasing CT values) from Days 0-3, and no signal at Day 4 for samples at 37ºC and 25ºC. However, samples held at 4ºC continued to give a positive PCR signal through Day 15, with no signal at Day 20. Assessment of viability by enrichment culture showed a sharp decline in viability within one day of incubation at 37ºC and 25ºC (Day 0 Ct= 12.2 vs Day 1 Ct= 23.5), and complete loss of culturability at Day 7. However, *S.* typhi cells held at 4ºC remained viable and culturable through Day 24, and lost culturability at Day 30. These results show that ST rapidly enters into a nonviable or viable-but-nonculturable state in sewage. Hence, detection of culturable ST in sewage may indicate the presence of an active infection or carrier state within the population, and detection of ST DNA in sewage can be used for burden of infection estimates.

1391

INTEGRATING PREVENTATIVE MEASURES OF COVID 19 THROUGH NEGLECTED TROPICAL DISEASES WASH & HEALTH PROMOTION: TANZANIA EXPERIENCE

Alistidia Simon[1], Cecilia Uisso[2], Oscar Kaitaba[1], Upendo Mwingira[3], Jeremiah Ng'ondi[3], Kimberly Kamara[4]

[1]*Ministry of Health, Dar es salaam, United Republic of Tanzania,* [2]*National Institute of Medical Research, Dar es salaam, United Republic of Tanzania,* [3]*RTI, Washington, DC, United States,* [4]*END FUND, New York, NY, United States*

Tanzania is endemic for Onchocerciasis, Lymphatic Filariasis, Trachoma, Schistosomiasis and Soil Transmitted Helminthiasis. Tanzania NTD programme and its partners implement various interventions including: Mass Drug Administration; morbidity management; advocacy for increased access to water sanitation and hygiene (WASH); and social behavioural change communication (SBCC) for transmission control and health promotion. MDA and, Case management through outreach approach and diseases specific surveys have been postponed following COVID -19 pandemic. Therefore, in order to sustain NTD prevention among communities, the program has planned to develop integrated preventive measures of COVID/NTDs. This is also in line with the World Health Organisation (WHO) guideline, which stipulates the NTD program through its WASH, SBCC and health promotion interventions. The NTD program will coordinate a technical working group to review NTD-WASH intervention measures to include COVID 19 prevention messages. Information education and communication/SBCC materials will be developed to integrate COVID 19 and NTDs preventive measures so that they can be circulated through different social medias and some will be printed for community distribution. The program will also join government efforts by purchasing protective gears for health providers. This presentation will report on the process, outputs, outcomes and impact of the integrated COVID 19 and NTDs preventive measures.

1394

SAFETY AND IMMUNOGENICITY OF COADMINISTRATION OF MENINGOCOCCAL TYPE A VACCINE WITH TYPHOID CONJUGATE VACCINE IN HEALTHY CHILDREN 15-23 MONTHS OF AGE IN BURKINA FASO

Sodiomon B. Sirima[1], Alphonse Ouédraogo[1], **Nouhoun Barry**[1], Mohamadou Siribié[1], Alfred Tiono[1], Issa Nébié Ouédraogo[1], Shrimati Datta[2], Yuanyuan Liang[2], Kathleen M. Neuzil[3], Matthew B. Laurens[3]

[1]*Groupe de Recherche Action en Santé (GRAS), Ouagadougou, Burkina Faso,* [2]*University of Maryland School of Medicine, Baltimore, MD, United States,* [3]*Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States*

The World Health Organization (WHO) recently pre-qualified a single-dose typhoid conjugate vaccine (Vi-TCV) for use in infants as young as six months of age. Recent testing of this vaccine in Nepalese children showed 82% efficacy. The WHO Strategic Advisory Group of Experts recommends studies of Vi-TCV co-administered with routine childhood vaccines in typhoid-endemic countries. We tested Vi-TCV co-administration with routine group A meningococcal conjugate (MCV-A) and measles-rubella (MR) vaccines at 15 months of age in Burkina Faso. We randomized 150 children in a 1:1:1 ratio to receive either Vi-TCV and control vaccine (inactivated polio) with a subsequent MCV-A vaccine 28 days later (Group 1), Vi-TCV and MCV-A (Group 2), or MCV-A and control vaccine (Group 3). To assess safety, we recorded local and systemic reactions for 28 days after immunization, with special attention for the third and seventh days. We assessed immunogenicity of Vi-TCV by ELISA and of MCV-A by serum bactericidal antibody (SBA) at days 0 and 28. Solicited symptoms associated with vaccination were similar for TCV and IPV, respectively at day 0 (fever 2.0% vs 5.9%; injection site pain 0% vs 2.0%), day 3 (fever 2.0% vs 3.9%; irritability 0% vs 2.0%; swelling 0% vs 2.0%), and day 7 (fever 4.0% vs 3.9%: irritability 2.0% vs 0%). Post-vaccination seroconversion for anti-Vi IgG antibody was similar for participants who received TCV with IPV vs TCV with MCV-A (93.8% vs 96.0%, p = 0.62). For participants who received MCV-A at enrollment, post-vaccination MCV-A antibody GMT was similar for participants who received MCV-A with TCV vs MCV-A with IPV (13,385 (95%CI 9784-18,311) vs 9410 (95%CI 6009-14,736), p=0.20), and a similar percentage achieved protective post-vaccination SBA titer >128 (100.0% vs 98.0%). Vi-TCV was well-tolerated and did not show a safety signal when co-administered with routine 15-month vaccination. Vi-TCV immunization resulted in a robust immune response without evidence of interference with MCV-A. Vi-TCV can be co-administered with MCV-A as part of routine childhood vaccination.

1395

SINGLE-DOSE, LIVE ORAL CHOLERA VACCINE CVD 103-HGR (PXVX0200) INDUCES LONG-TERM SERUM VIBRIOCIDAL ANTIBODIES IN ADOLESCENTS

James M. McCarty[1], Lisa Bedell[2], Sean Bennett[2]

[1]*Stanford University, Stanford, CA, United States,* [2]*Emergent BioSolutions, Gaithersburg, MD, United States*

The attenuated recombinant *Vibrio cholerae* O1 strain CVD 103-HgR, redeveloped as PVXV0200, elicits a rapid SVA response and protects against cholera-induced diarrhea in adult volunteer challenge trials. Previous studies documented persistence of SVA seroconversion, defined

as a four-fold or higher rise from baseline, elevated geometric mean titers (GMTs) and geometric mean fold increase (GMFI) at 180 days post immunization with PXVX0200. Methods: In a phase 4, placebo-controlled, double-blind, multi-center study performed to assess the safety and immunogenicity of a single oral dose of PXVX0200 in children and adolescents aged 2-17 years, volunteers were randomized 6:1 to receive 1 x 10^9 colony forming units of PXVX0200 or placebo. Immunogenicity endpoints included SVA seroconversion rates, GMTs and GMFI on days 1, 11, 29 (ages 2-17) and 91, 181 (ages 12-17). In a subset aged 12-17 years SVA, GMTs and GMFI were also assessed on days 365, 547, and 730. Results: A total of 72 adolescents were enrolled in the long-term sub-study. GMTs and GMFI remained elevated above baseline and in most subjects SVA seroconversion persisted for 2 years after vaccination. Immunogenicity of PXVX0200 and Placebo ont face=»Times New Roman» size=»3»> Conclusion: Vaccination with PXVX0200 produced a robust immune response which persisted for 2 years in adolescents. Since SVA seroconversion is a strong correlate of protection, PXVX0200 should protect against cholera infection for at least 2 years when traveling to or visiting at risk countries. Long-term studies of cholera-specific memory B-cells are in progress and will further characterize persistence of immunity.

1396

ONE DOSE ORAL CHOLERA VACCINE COVERAGE DURING AN OUTBREAK IN URBAN HARARE, ZIMBABWE, 2018

Ashley T. Longley[1], Nandini Sreenivasan[2], Emmaculate Lebo[3], Aluwiso Mukavhi[4], Grace Chaora[4], Trymore Chawurura[5], Maxwell Rupfutse[3], Lloyd Machacha[5], Manes Munyanyi[5], Jethro Chakauya[3], Mayuko Takamiya[6], Naeema Logan[2], Marc Poncin[3], Kashmira Date[2], Portia Manangazira[5]

[1]*CDC Foundation, Atlanta, GA, United States,* [2]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [3]*World Health Organization, Harare, Zimbabwe,* [4]*Zimbabwe National Statistics Agency, Harare, Zimbabwe,* [5]*Ministry of Health and Child Care, Harare, Zimbabwe,* [6]*Centers for Disease Control and Prevention, Zimbabwe Country Office, Harare, Zimbabwe*

In September 2018, the Zimbabwe Ministry of Health and Child Care (MOHCC) declared a cholera outbreak, with 98% of cases reported from Harare City. In October 2018, the MOHCC conducted the country's first oral cholera vaccine (OCV) campaign. A total of 1,351,808 persons aged ≥1 year in 16 suburbs of Harare Province were targeted with OCV using fixed sites, mobile teams, and school-based vaccination. We estimated OCV coverage after the first dose to help inform planning for the second dose campaign to be conducted in March-April 2019. We conducted a two-stage cluster survey to estimate OCV coverage by age group (1-4 years, 5-14 years, and ≥15 years). We selected 40 of 3170 clusters by probability proportionate to size using 2012 census data; 30 households were systematically sampled in each cluster. In households with an eligible individual, we completed one household questionnaire and interviewed all eligible individuals in each age group. Information on OCV receipt was obtained from vaccination cards or verbal report. Coverage estimates and 95% confidence intervals were calculated. Overall, 855 household and 2,355 individual interviews were conducted. One-dose OCV coverage was 71% (95% CI: 67%-76%): 75% (95% CI: 69%-81%) among children 1-4 years old, 87% (95% CI: 84%-90%) among children 5-14 years old, and 65% (95% CI: 58%-71%) among those ≥15 years old. OCV vaccination coverage was significantly higher among females than males ≥15 years old (71% vs. 55%, p<0.0001). The most common reasons for non-vaccination were absence during the campaign (41%) and lack of awareness of the campaign (14%). OCV use is an important component of a multisectoral cholera control strategy along with improvements in water, sanitation, and hygiene. School-based vaccination was a successful strategy to deliver OCV to school-aged children 5-14 years old in Zimbabwe. Our results contribute important data on the use of single dose OCV in outbreak settings and highlight the continuing need to explore communication and delivery strategies to improve coverage among adult males and children outside of the routine vaccination age group.

1397

PERSISTENCE OF IMMUNOGLOBULIN M ANTIBODIES AFTER VACCINATION WITH LIVE ATTENUATED JAPANESE ENCEPHALITIS VACCINE

Susan L. Hills[1], Alex Van Keulen[1], Jodi Feser[2], Amanda Panella[1], G William Letson[2], J Erin Staples[1], Anthony A Marfin[2], Aaron C. Brault[1]

[1]*Centers for Disease Control and Prevention, Fort Collins, CO, United States,* [2]*PATH, Seattle, WA, United States*

Japanese encephalitis (JE) is a vaccine-preventable, mosquito-borne disease found in Asia and parts of the western Pacific. Substantial progress with JE control has been made during the past decade with most endemic countries now having JE vaccination programs. Most countries use the live attenuated SA14-14-2 JE vaccine (CD-JEV). Following vaccination with CD-JEV, limited data suggest JE IgM antibodies are detectable in serum in >10% children for at least 4 weeks, but duration of IgM persistence is unknown. Some other live attenuated flavivirus vaccines elicit prolonged IgM antibodies; for example, 73% of yellow fever vaccinated individuals have IgM antibodies 3-4 years after vaccination. If a child develops encephalitis during the weeks to months following CD-JEV vaccination, and JE IgM is detected in serum, the question arises if this is a wild-type JE virus infection indicating vaccine failure or reflects post-vaccination IgM persistence. To better understand JE IgM seropositivity following vaccination, serum samples from a previous CD-JEV study among Sri-Lankan infants were tested to determine frequency of JE IgM antibodies on days 28, 180, and 365 post-vaccination. We used the CDC in-house enzyme-linked immunosorbent assay (ELISA) and the Inbios JE Detect™ ELISA. Among 258 day 28 samples tested with the CDC ELISA, 46 (18%) were IgM positive, 64 (25%) were equivocal, 5 (2%) were non-specific, and 143 (55%) were negative; eight (3%) samples subsequently had positive or equivocal results on day 180 and one each was equivocal or positive on day 365. With the Inbios ELISA, 75 (29%) samples were positive, 43 (17%) were equivocal, and 140 (54%) were negative on day 28; three (1%) samples were positive or equivocal on day 180 and one was positive on day 365. Overall, we found that >40% of infants might have IgM positive or equivocal results within a month of vaccination but <5% are likely to have any detectable IgM antibodies by 6 months. Our results will help healthcare workers determine whether the presence of JE IgM antibodies in serum of a child with encephalitis in the weeks to months after CD-JEV vaccination are likely vaccine-related.

1398

UNPLANNED HEALTHCARE DURING TRAVEL: A DESCRIPTIVE ANALYSIS FROM THE GEOSENTINEL NETWORK

Lin H. Chen[1], Watcharapong Piyaphanee[2], Marta Diaz-Menendez[3], Hilmir Asgeirsson[4], Philippe Gautret[5], Elizabeth Barnett[6], Michael Libman[7], Patricia Schlagenhauf[8], Karin Leder[9], Katherine Plewes[10], Kunjana Mavunda[11], Martin Grobusch[12], Davidson Hamer[13]

[1]*Mount Auburn Hospital and Harvard Medical School, Cambridge, MA, United States,* [2]*Mahidol University Faculty of Tropical Medicine, Bangkok, Thailand,* [3]*Hospital La Paz Carlos Iii, Madrid, Spain,* [4]*Karolinska University Hospital, Stockholm, Sweden,* [5]*Aix Marseille University, Marseille, France,* [6]*Boston Medical Center, Boston, MA, United States,* [7]*McGill University J.D. MacLean Centre for Tropical Diseases, Cambridge, QC, Canada,* [8]*University of Zurich, Zurich, Switzerland,* [9]*Monash University and Royal Melbourne Hospital, Melbourne, Australia,* [10]*University of British Columbia, Vancouver, BC, Canada,* [11]*International Travel Clinic, Miami, FL, United States,* [12]*University of Amsterdam, Amsterdam, Netherlands,* [13]*Boston University School of Public Health and Boston University School of Medicine, Boston, MA, United States*

Characteristics and outcomes of travelers who seek care for medical problems arising during travel are not well described. Travelers evaluated at GeoSentinel Surveillance Network sites who sought healthcare during travel were eligible for inclusion. Data were collected on presenting illness,

characteristics of the healthcare provided, and outcomes of treatment. Among 36,175 travelers evaluated at GeoSentinel sites February 2018 through September 2019, 1274 (3.5%) reported receiving healthcare abroad during travel. Median age was 35 years (range 0-84); 52.7% were female. Over half (56.3%) traveled for tourism, followed by visiting friends and relatives (19.9%), missionary/humanitarian/volunteer/community service (7.1%), and occupational (7.0%). Only 17% reported pretravel encounters. Injury accounted for 24.9% of visits; 74.6% were for other medical problems. Travelers presented to hospitals (47.5%) or out-patient clinic/private doctor's offices (34.8%); 0.5% sought dental care. Thailand, India and Indonesia were the most common countries where travelers in our analysis received healthcare. Gastrointestinal and respiratory system diagnoses were reported frequently (n=219, 17.2% of total; and n=58, 4.6% of total, respectively). Excluding animal exposure/post-exposure prophylaxis (n=377;29.1%), top specific diagnoses acquired in contact with healthcare during travel were malaria (12.2%), acute diarrhea (9.9%), and dengue (5.4%). Two-thirds (66.6%) of travelers improved or resolved completely, 29.7% of travelers were unimproved, 3.8% deteriorated or developed complications; no deaths were reported. Travelers who sought healthcare during travel often had conditions for which pre-travel consultation could provide vaccinations and advice, including avoiding animal bites or receiving rabies pre-exposure prophylaxis, use of malaria prophylaxis, self-treatment of diarrhea, and injury prevention.

1399

EARLY DIAGNOSIS AND FOLLOW-UP OF ACUTE SCHISTOSOMIASIS IN A COHORT OF BELGIAN TRAVELLERS USING ANTIBODY AND CIRCULATING ANODIC ANTIGEN (CAA) DETECTION METHODS

Pytsje Hoekstra[1], Marjan van Esbroeck[2], Claudia J. de Dood[3], Paul L. Corstjens[3], Lieselotte Cnops[2], Christel J. Zeijl - van der Ham[4], Jutte J. de Vries[4], Govert J. van Dam[1], Joannes Clerinx[5], Lisette van Lieshout[1]

[1]*Department of Parasitology, Leiden University Medical Center, Leiden, Netherlands,* [2]*Department of Clinical Sciences, Institute of Tropical Medicine Antwerp, Antwerp, Belgium,* [3]*Department of Cell and Chemical Biology, Leiden University Medical Center, Leiden, Netherlands,* [4]*Department of Medical Microbiology, Leiden University Medical Center, Leiden, Netherlands,* [5]*Department of Clinical Sciences, Institute of Tropical Medicine Antwerp, Leiden, Netherlands*

Diagnosing acute schistosomiasis in travellers can be challenging as clinical symptoms might appear before egg production has started or before specific antibodies can be detected. *Schistosoma* circulating antigens, already detectable a few weeks after infection, seem to be a promising diagnostic alternative. Here, we evaluated the detection of urine and serum schistosome Circulating Anodic Antigen (CAA) in comparison to antibody-based serology assays in a group of recently exposed and infected Belgian travellers, before and after praziquantel (PZQ) treatment. Samples were available from a cohort of 34 travellers with a serum PCR-confirmed *Schistosoma* infection as early as 4 to 5 weeks after exposure during a holiday in South Africa. Pre- and 6 to 7 weeks post-treatment samples were subjected to an in-house ELISA and IFA assay (serum), as well as to the ultra-sensitive and highly specific UpConverting Phosphor labelled Lateral Flow (UCP-LF) CAA test (serum and urine). *Schistosoma* antibodies were detected on at least one occasion in 29/34 (85%) of the travellers, with no changes after treatment. Urine CAA concentrations were detected in 13/34 (38%) individuals pre-treatment, and these travellers all became negative after treatment. Serum CAA was positive in 33/34 (97%) travellers pre-treatment, while all except one became serum CAA negative post-treatment. Based on the in-house antibody assays almost all travellers were positive, which is a better performance than the already previously described performance of commercial antibody tests on the same sample set. As expected, all remained antibody positive after treatment, confirming the inherent limitations of serology for monitoring treatment efficacy. Serum CAA was demonstrated in almost all travellers and levels declined rapidly after PZQ treatment. Therefore, compared to either commercial or in-house antibody assays, serum PCR or urine CAA, the serum CAA test seems to be the most sensitive method for detecting early *Schistosoma* infections and for monitoring the effect of PZQ treatment in recently exposed travellers.

1400

CLINICAL EVALUATION OF THE FILMARRAY GLOBAL FEVER PANEL

Brian W. Jones, David A. Rabiger, Mark A. Gurling, Madeline Veloz, Olivia Jackson, Marissa Burton, Natalie Batty, Ashley Wiltsie, Haley Halberg, Pascal Belgique, Cynthia D. Andjelic, Cynthia L. Phillips

BioFire Defense, LLC, Salt Lake City, UT, United States

Acute Febrile Illness (AFI) is caused by a diverse set of pathogens. Identifying the causal agent is often slow and difficult. The FilmArray Global Fever (GF) Panel, developed by BioFire Defense in collaboration with the U.S. Department of Defense and NIAID, uses an automated, multiplex nested PCR system to evaluate whole blood samples for multiple pathogens simultaneously in under an hour. BioFire Defense conducted a prospective clinical study to evaluate the positive percent agreement (PPA) and negative percent agreement (NPA) of the GF Panel when used to test blood collected from subjects who have recently had fever. The results of this study will be reported in two submissions to the US FDA. Eleven locations around the world tested 1,865 specimens on the GF Panel. The rate of positive detections was 35% (652/1865), with *Plasmodium* spp. accounting for the majority of positives (53.4%, 348/652) and dengue virus the second most (40.5%, 264/652). Other detected pathogens include *Leptospira* spp., West Nile virus, Zika virus, *Leishmania* spp., Crimean-Congo hemorrhagic fever virus, and chikungunya virus. Twenty-eight (28) specimens had more than one detected pathogen (4.3% of positive specimens). Comparator testing consisted of in-house developed PCR assays followed by bidirectional sequencing. PPA between GF Panel and comparator testing ranged between 92.7-100%, and the NPA ranged between 99.3-100%. In all cases, discrepancies coincided with analytes that were near the limit of detection of the GF Panel and comparator assays. When the GF Panel result was compared to site-specific malaria testing (e.g., thick/thin smear), the PPA ranged between 94.7-100% and the NPA ranged between 43.3-100%. Analysis of the NPA suggests that the GF Panel is more sensitive than microscopy, producing "discrepancies" for this comparison. The wide range in NPA between sites could be due to variation in microscopy technique; the GF Panel eliminates such variation because it is fully automated. The results show that the FilmArray GF Panel could aid in rapid and actionable AFI diagnosis caused by multiple, sometimes co-occurring, pathogens.

1401

LIVE-ATTENUATED CHIMERIC VACCINE CANDIDATES AGAINST ZIKA VIRUS INDUCE PROTECTIVE IMMUNITY IN AG129 MICE AND NON-HUMAN PRIMATES

Whitney Baldwin[1], Holli Giebler[1], Janae Stovall[2], Ginger Young[3], Kelly Bohning[3], Hansi Dean[3], Jill Livengood[1], Claire Huang[2]

[1]*Takeda Vaccines; Centers for Disease Control and Prevention/Division of Vector-Borne Diseases, Fort Collins, CO, United States,* [2]*Centers for Disease Control and Prevention/Division of Vector-Borne Diseases, Fort Collins, CO, United States,* [3]*Takeda Vaccines, Cambridge, MA, United States*

The recent global spread of Zika virus (ZIKV) and association with congenital birth defects in children born to mothers infected with ZIKV during pregnancy has prompted the development of numerous vaccine platforms for the prevention of congenital ZIKV syndrome (CZS). Utilizing the replicative backbone of the attenuated dengue virus 2 (DENV-2) PDK-53 vaccine virus and the pre-membrane (prM) and envelope (E) genes of ZIKV, we have developed several chimeric (DENV-2/ZIKV), live-attenuated vaccine (LAV) candidates against ZIKV. Characterization to date indicate these vaccine candidates are: 1) unlikely to be transmitted by *Aedes* mosquitoes, 2) highly attenuated in numerous *in vitro* and *in vivo* models, 3) immunogenic and protective against lethal ZIKV challenge in

an interferon-receptor deficient AG129 mouse model. Utilizing the AG129 mouse model for additional safety and efficacy analysis, we show that these vaccine candidates do not replicate in the male reproductive tract and are not sexually transmitted to female mice. In addition, vaccinated dams challenged by both vertical and sexual transmission routes do not display maternal viremia or detectable fetal viral tissue loads. Additionally, we have extended our studies to assess the safety, immunogenicity and protective capacity of our vaccine candidates in non-human primates (NHPs). Indian rhesus macaques immunized with DENV-2/ZIKV LAV candidates showed no clinical signs of disease due to immunization and dermal Draize scoring revealed no edema or erythema at inoculation sites. Additionally, vaccine viremia equivalency measured by ZIKV E gene qRT-PCR RNA quantification was low or undetectable through up to 14 days post-immunization. Collectively, these data indicate that these vaccine candidates are well attenuated and have an acceptable safety profile in NHPs. Importantly, a single immunization elicited a rapid ZIKV-neutralizing antibody response and conferred complete protection upon challenge with ZIKV PRVABC59 six months post-primary immunization.

1402

A PHASE 1 DOSE RANGING TRIAL OF A ZIKV MRNA VACCINE CANDIDATE IN HEALTHY FLAVIVIRUS BASELINE SEROPOSITIVE AND SERONEGATIVE ADULTS

Nancy Le Cam, Veronica Faughnan, Rolando Pajon, Shiva Kalidindi, Shu Han, Wellington Sun, Tal Zaks, Hamilton Bennett

ModernaTx, Cambridge, MA, United States

Zika virus (ZIKV) is a public health concern due to the risk of severe clinical complications. mRNA-1893 is an investigational Zika mRNA vaccine currently in Phase 1. The mRNA encodes the pre-membrane and envelope (prME) structural proteins of ZIKV and is encapsulated in a lipid nanoparticle (LNP). The Phase 1 randomized, observer blinded, placebo-controlled study enrolled 120 participants to one of four dose level cohorts (10µg, 30µg, 100µg, 250µg). Among the 30 participants per cohort, 25 are initially flavivirus infection-naïve (seronegative) and 5 have pre-existing flavivirus antibodies (seropositive). The primary objective is to evaluate the safety, tolerability and reactogenicity of mRNA-1893 administered as a 2-dose regimen given 28-days apart; secondary objectives include assessment of the ZIKV-specific neutralizing antibody (nAb) titers as measured by Plaque Reduction Neutralization Test (PRNT) and Microneutralization assay (MN). An interim analysis of the study reports safety and immunogenicity data from the 10µg and 30µg cohorts are available. Both dose levels were generally well-tolerated, and there were no vaccine-related serious adverse events (SAEs) or adverse events of special interest (AESI). The most frequent solicited adverse event was local pain at the injection site. mRNA-1893 induced a strong nAb response in both seronegative and seropositive. In the seronegative group, seroconversion rates, defined as the change in titers from below the Lower Limit of Quantification (LLOQ) to equal or above the LLOQ, after the second vaccination was 94.4% in the 10µg dose level and 100% in the 30µg dose level, based on the $PRNT_{50}$. In the flavivirus-seropositive group, seroconversion rates, defined as a 4-fold increase in titers, after the second vaccination reached 50% in the 10µg dose level and 75% in the 30µg dose level, based on the $PRNT_{50}$. For all participants receiving mRNA-1893, independent of serostatus at baseline, the seroconversion rate was 86.4% (95%CI: 65.1, 97.1%) for the 10µg cohort and at 95.5% (77.2, 99.9%) for the 30µg. MN data were consistent with $PRNT_{50}$ data. Results from all 4 Cohorts will soon be available.

1403

RESULTS OF A PHASE 1 STUDY TO EVALUATE SAFETY AND PHARMACOKINETICS OF ANTI-ZIKA VIRUS IMMUNE GLOBULIN (HUMAN) (ZIKV-IG) IN HEALTHY VOLUNTEERS

Jane White[1], Priya Tunga[2], Debbie Anderson[3], Ken C. Iledan[3], Tobi Loreth[3], Geraldine Parrera[3], Hugo Astacio[3], Bojan Drobic[2], **Jason S. Richardson**[3]

[1]*Emergent BioSolutions Inc, New York, NY, United States,* [2]*Emergent BioSolutions Inc, Toronto, ON, Canada,* [3]*Emergent BioSolutions Inc, Winnipeg, MB, Canada*

Zika virus (ZIKV) is primarily transmitted through the bite of infected *Aedes aegypti* or *Ae. albopictus* mosquito species. ZIKV infection during pregnancy has been linked to adverse fetal/infant outcomes including microcephaly, brain anomalies, ocular disorders, intrauterine growth restriction, and other congenital malformations. ZIKV-IG (Anti-ZIKV Immune Globulin [Human]) is being developed for prophylaxis of ZIKV in at-risk populations including women of childbearing potential and pregnant women. ZK-001 was a Phase 1, double-blind, randomized, placebo-controlled study [ClinicalTrials.gov Identifier: NCT03624946] conducted in Canada from June 2018 to March 2019, to assess the safety and pharmacokinetics (PK) of a single 50 mL ZIKV-IG (4.65 g of IgG) dose by IV infusion to healthy adult male or non-pregnant female subjects 18-55 years of age with blood type O+/-. Subjects received either ZIKV-IG (n=19) or saline placebo (n=11). Safety was evaluated based on adverse events (AEs), laboratory test results, physical exams and vital signs. Blood samples were collected at baseline and post-dose at 1, 3, 8, 24 h (Day 2), Days 3, 4, 6, 8, 10, 12, 15, 22, 29, 43, 57 and 85 for PK analysis (Luminex binding immunoassay, [neutralizing] against ZIKV Envelope protein). Of the AEs considered treatment-related, 3 events of headache (mild) were reported in 3 of 19 (15.8%) subjects in the ZIKV-IG group. There were no serious AEs, no reported deaths and no discontinuations due to AEs in either study group. The PK characteristics of ZIKV-IG in this study were; C_{max}, 182.3 (0.2; CV %) U/mL; T_{max}, 2.3 (1.0; arithmetic mean [standard deviation]) h; $AUC_{0-\infty}$, 77224 (0.2, CV %) h*U/mL and; half-life, 28.1 days. Overall, the safety profile of ZIKV-IG in a population of healthy adult subjects with blood type O+/- appeared safe and well-tolerated. The ZIKV-IG half-life of 28 days was similar to that of commercially available administered IV immunoglobulins at similar concentrations in healthy adults. The C_{max}, T_{max}, AUC, and half-life of ZIKV-IG were also consistent with these PK parameters for human-derived IgG products manufactured with EBCI's hyperimmune platform.

1404

CD8+ T CELLS MEDIATE AN NS3-BASED VACCINE PROTECTION AGAINST ZIKA VIRUS: A NEW STRATEGY FOR VACCINE DEVELOPMENT

Annie Elong-Ngono[1], Thasneem Syed[1], Anh-Viet Nguyen[1], Jose Angel Regla-Nava[1], Mercylia Susantono[1], Darina Spasova[2], Allison Aguilar[2], Melissa West[2], Jessica Sparks[2], Andrew Gonzalez[1], Emilie Branche[1], Jason L DeHart[2], Jerel Boyd Vega[2], Priya Prakash Karmali[3], Padmanabh Chivukula[3], Parinaz Aliahmad[2], Nathaniel Wang[2], Sujan Shresta[1]

[1]*La Jolla Institute for Immunology, La Jolla, CA, United States,* [2]*Synthetic Genomics, Inc, La Jolla, CA, United States,* [3]*Arcturus Therapeutics, San Diego, CA, United States*

Zika virus (ZIKV), a member of the flaviviridae family, is associated with severe congenital malformations in infants born to infected mothers, and with neurological disorders, such as Guillain-Barré syndrome, in infected adults. To date, the development of anti-flavivirus vaccines has focused predominantly on the induction of neutralizing antibodies. Paradoxically, a suboptimal antibody response may enhance disease severity upon subsequent flaviviral infection through a phenomenon known as antibody-dependent enhancement. Thus, alternative approaches to ZIKV vaccine design are necessary. Here, we report induction of a protective anti-ZIKV CD8+ T cell response in the HLA-B*0702 *Ifnar1*$^{-/-}$ transgenic

mouse model using an alphavirus-based replicon RNA vaccine expressing ZIKV nonstructural protein NS3, a potent T cell antigen. The NS3 vaccine did not induce neutralizing antibody response, but elicited cytotoxic and polyfunctional CD8+ T cells that were necessary and sufficient for controlling viral burden and preventing death in lethally infected adult mice. In addition, the NS3 vaccine prevented fetal growth restriction and death in infected pregnant mice. These data provide proof of concept that CD8+ T cells, major mediators of the ZIKV NS3 vaccine induce protection and suggest a new strategy to develop safe and effective anti-flavivirus vaccines that avoid inadvertent antibody-dependent enhancement and provide strong protection. Vaccines will play a critical role in tackling the global infectious disease problem.

1405

ANTIGEN-SPECIFIC T CELLS RESTRICT VIRAL DIVERSITY AND DISSEMINATION DURING ZIKA VIRUS INFECTION

Mariah Hassert[1], Christopher M. Weiss[2], Lark L. Coffey[2], Amelia K. Pinto[1], James D. Brien[1]

[1]*Saint Louis University, St. Louis, MO, United States,* [2]*University of California-Davis, Davis, CA, United States*

Due to high mutation rates and selective pressures, RNA viruses such as Zika virus (ZIKV) do not replicate with a single genomic sequence, but rather as an amalgam of related sequences. Increased diversity of the viral swarm has been linked to increased virulence and dissemination, and as such, it is imperative to understand the factors which influence viral population dynamics within the host. We previously demonstrated that antigen specific CD4+ and CD8+ T cells play a critical role in restriction of viral replication in the CNS and periphery during ZIKV infection in mice. We hypothesize that as a result of their significant restriction on replication, T cells play an important role in limiting ZIKV quasispecies diversity. To this end, we depleted CD4+ and CD8+ T cells from type 1 interferon receptor deficient mice prior to ZIKV infection. We sampled blood at multiple times post-inoculation, and from the spleens and brains at the peak of viral burden in the periphery and CNS. We deep sequenced ZIKV from these samples using an amplicon-based Illumina approach (PrimalSeq) and used the accompanying analytics software (iVar) to detect single nucleotide variants (SNVs). We found that at days corresponding to the peak of the T cell response, mice deplete of T cells had significantly higher viral RNA levels in the periphery and CNS. Moreover, in the absence of T cells, a more diverse population of SNVs were found. We are currently analyzing specific SNVs in the T cell-depleted animals and the implications of their development on pathogenesis and dissemination. These studies integrating immunology, virology, and evolution link immune- restricting phenotypes to distinct outcomes for the virus, and provide a more thorough understanding of T cell restriction of viral replication and its impact on viral emergence.

1406

ASSESSING ANTIVIRAL FUNCTIONS OF A ZIKV-NEUTRALIZING HUMAN IGM AS A CANDIDATE FOR ANTIBODY-BASED PROPHYLAXIS DURING PREGNANCY

Tulika Singh[1], Kwan Ki-Hwang[1], Rebecca Jones[1], Joshua Eudailey[1], Helen Webster[1], Cesar Lopez[2], Premkumar Lakshmanane[2], Kan Luo[1], Robert J. Edwards[1], Camila Giuberti[3], Summer Zhang[4], Morgan Gladden[1], Jesse Mangold[1], Joshua Tu[1], Maria Dennis[1], Reynaldo Dietze[3], Aravinda de Silva[2], Helen Lazear[2], Eng Eong Ooi[4], Sallie Permar[1], Mattia Bonsignori[1]

[1]*Duke University, Durham, NC, United States,* [2]*University of North Carolina – Chapel Hill, Chapel Hill, NC, United States,* [3]*Universidade Federal do Espírito Santo, Vitoria, Brazil,* [4]*Duke University-National University of Singapore Medical School, Singapore, Singapore*

Congenital transmission of Zika virus (ZIKV) can produce a spectrum of neurodevelopmental defects in ~7-14% of infants born to ZIKV-infected mothers. With no licensed ZIKV vaccine available, passive administration of immunoglobulins provides a valuable prophylactic option in pregnancy. We isolated circulating ZIKV-specific memory B cells from 4 Brazilian women with ZIKV infection during pregnancy. Individually cultured memory B cells yielded 97 monoclonal antibodies (mAbs), of which 45 IgG, 2 IgM, and 2 IgA bound ZIKV virions (>mean + 2SD no sample). From a ZIKV+/IgM+ memory B cell, we established a human B cell line (DH1017) via EBV transformation and purified mAb DH1017.IgM. This mAb was isolated from the memory B cells of a pregnant woman with prolonged viremia (~40 days) at an early convalescent timepoint (30 days post viremia). Next, we recombinantly produced mAb DH1017.IgG, which retained the same IgV(D)J but with the IgG Fc, to assess impact of mAb isotype on antibody functions. Compared to DH1017.IgG, DH1017.IgM bound to ZIKV 6-fold better (ED50 = 3000 pM and 474 pM, respectively) and neutralized 39-fold more potently (FRNT50 = 996 pM and 25 pM, respectively). Neither DH1017.IgM nor DH1017.IgG bound or neutralized dengue virus (DENV) serotypes 1-4. Addition of complement from human serum enhanced neutralizing activity of both mAbs in a dose-dependent manner. Thus, we have isolated a ZIKV type-specific and strongly neutralizing IgM mAb that is more potent than its IgG counterpart. The dramatic increase in potency from the IgG to IgM form of DH1017 suggests that IgM-secreting memory B cells and ZIKV IgM may play an important role in protection against ZIKV infection. Unlike IgG, IgM is not transferred across the placenta. This means that this mAb would not facilitate fetal disease by transcytosis of ZIKV, nor increase risk of antibody-mediated severe DENV infection of the newborn. These characteristics posit DH1017.IgM as a suitable candidate for a prophylactic ZIKV intervention during pregnancy.

1407

PRIOR DENGUE IMMUNITY MAY ENHANCE ZIKA VIRUS INFECTION IN THE PLACENTA IN NON-HUMAN PRIMATES

Chelsea M. Crooks[1], Andrea M. Weiler[2], Sierra L. Rybarczyk[2], Mason I. Bliss[2], Anna S. Jaeger[3], Megan E. Murphy[4], Heather A. Simmons[2], Jennifer M. Hayes[2], Andres Mejia[2], Michael K. Fritsch[5], Elizabeth A. Brown[1], Katarina M. Braun[1], Ann M. Mitzey[4], Elaina Razo[6], Keisuke Yamamoto[5], Phoenix M. Shepherd[5], Amber R. Possell[2], Kara Weaver[2], Terry K. Morgan[7], Christina M. Newman[5], Dawn M. Dudley[5], Nancy Schultz-Darken[2], Eric Peterson[2], Leah C. Katzelnick[8], Angel Balmaseda[9], Eva Harris[8], Emma L. Mohr[6], Thaddeus G. Golos[4], David H. O'Connor[5], Matthew T. Aliota[3], Thomas C. Friedrich[1]

[1]*Department of Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI, United States,* [2]*Wisconsin National Primate Research Center, University of Wisconsin-Madison, Madison, WI, United States,* [3]*Department of Veterinary and Biomedical Sciences, University of Minnesota, Twin Cities, St. Paul, MN, United States,* [4]*Department of Comparative Biosciences, University of Wisconsin-Madison, Madison, WI, United States,* [5]*Department of Pathology and Laboratory Medicine, University of Wisconsin-Madison, Madison, WI, United States,* [6]*Department of Pediatrics, University of Wisconsin-Madison, Madison, WI, United States,* [7]*Departments of Pathology and Obstetrics and Gynecology, Oregon Health and Science University, Portland, OR, United States,* [8]*Division of Infectious Diseases and Vaccinology, University of California Berkeley, Berkeley, CA, United States,* [9]*Laboratorio Nacional de Virología, Centro Nacional de Diagnóstico y Referencia, Ministry of Health, Managua, Nicaragua*

Both Zika virus (ZIKV) and dengue virus (DENV) are transmitted by *Aedes spp.* mosquitoes and are therefore often co-endemic in the same geographic locations. Cocirculation of these viruses has led to concerns about antibody-dependent enhancement (ADE) due to the homology between ZIKV and DENV and the observation of ADE among DENV serotypes. While antigenic interactions between ZIKV and DENV have been studied in several models, no study has examined the impact of prior DENV immunity on ZIKV pathogenesis during pregnancy in a non-human primate model. To address this, we used our established rhesus macaque model, which closely mimics the reproductive physiology of humans. Eight non-pregnant macaques were inoculated subcutaneously (sc) with 10^4 PFU of DENV-2/US/BID-V594/2006, a low-passage DENV-2 isolate from Puerto Rico. Following confirmation of productive DENV infection and clearance, macaques were bred, and 3-4 months following DENV infection and

pregnancy confirmation, inoculated sc with 10^4 PFU of ZIKV-PRVABC59 during the mid-first trimester (~gestation day 45; full term is 165±10 days). ZIKV infection was monitored by plasma qRT-PCR and fetal growth was monitored by weekly ultrasound. All eight pregnancies progressed without adverse outcomes and no gross fetal abnormalities were noted at delivery. No significant differences were observed in the peak, duration, or area under the curve of ZIKV viremia when compared to a group of five DENV-naïve, ZIKV-infected pregnant macaques. A set of ~50 maternal-fetal interface (MFI) tissue samples were collected from each animal at delivery. QRT-PCR detected ZIKV RNA >10 copies/mg in tissues from N=5/8 DENV-exposed macaques, whereas RNA was detected in tissues from only N=1/4 DENV-naïve macaques. Our data do not support a role for prior DENV exposure in modulating the severity of fetal outcomes in ZIKV infection. Ongoing analyses are examining the relationship between antibody titers, duration of viremia, and a potential increase in ZIKV infection of the MFI in DENV-exposed macaques.

1408

THE TOP 1% - QUANTIFYING THE UNEQUAL DISTRIBUTION OF MALARIA: THE CASE OF BRAZIL

Narimane Nekkab[1], Raquel Lana[2], Andre Siqueira[2], Cassio Peterka[3], Paola Marchesini[4], Marcus Lacerda[3], Ivo Mueller[1], Michael White[1], Daniel Villela[2]

[1]*Institut Pasteur, Paris, France,* [2]*Fundação Oswaldo Cruz, Rio de Janeiro, Brazil,* [3]*Fundação de Medicina Tropical Dr. Heitor Vieira Dourado, Manaus, Brazil,* [4]*Department of Transmissible Diseases Surveillance, Ministry of Health, Brasilia, Brazil*

As malaria endemic countries strive towards elimination, intensified spatial heterogeneities of local transmission could undermine the effectiveness of traditional intervention policy. We explore the dynamic nature of large-scale and long-term malaria heterogeneity by exploiting Brazil's rich clinical malaria reporting database from 2004 to 2018 of 6 million reported cases. We adapted econometric methods for understanding wealth inequality for malaria data to assess targeting of health system resources. As transmission declined, heterogeneity increased with cases clustering into smaller subpopulations across the territory. In 2004, the 1% of health units with the greatest number of cases accounted for 46% of all reported *P. vivax* cases, whereas in 2018 52% of *P. vivax* cases occurred in the top 1% of health units. *P. falciparum* had lower levels of transmission than *P. vivax*, and also had greater levels of heterogeneity with 75% of cases occurring in the top 1% of health units. Age and gender stratification of cases revealed peri-domestic and occupational exposure settings that remained relatively stable. The pathway to decreasing incidence is characterized by higher proportions of cases in males, in adults, due to importation, and due to *P. vivax*. Characterization of spatio-temporal heterogeneity and risk groups can aid stratification for improved malaria control towards elimination with increased heterogeneity potentially allowing for more efficient and cost-effective targeting. Although distinct epidemiological phenomena were clearly observed as malaria transmission declines, we argue that there is no canonical path to malaria elimination and a more targeted and dynamic surveillance will be needed if Brazil decides to adopt the elimination target.

1409

ASSESSING MULTI-SCALE EXPOSURE PATHWAYS IN ZONES OF PATCHY DISTRIBUTION OF MALARIA RESERVOIRS WITHIN REMOTE REGIONS OF MYANMAR

Tatiana Loboda[1], Amanda Hoffman-Hall[1], Dong Chen[1], Allison Baer[1], Varada Shevade[1], Robin Puett[1], Julie Silva[1], Kay Thwe Han[2], Kyin Hla Aye[2], Zay Yar Han[3], Thura Htay[4], Zaw Win Thein[4], Poe Poe Aung[4], Christopher Plowe[3], Myaing Myaing Nyunt[3]

[1]*University of Maryland, College Park, MD, United States,* [2]*Department of Medical Research, Yangon, Myanmar,* [3]*Duke Global Health Institute, Durham, NC, United States,* [4]*Duke Global Health Institute, Myanmar, Yangon, Myanmar*

Over the past decade Myanmar has made tremendous strides towards achieving the goal of national malaria elimination by 2030, contributing to the World Health Organization's global malaria eradication agenda. However, in recent years the progress has stalled despite the rising urgency of eliminating artemisinin-resistant malaria from countries in the Greater Mekong Subregion, including Myanmar. Although clinical malaria burden is declining, the increasing recognition of disparate pockets of clinically-silent malaria in remote parts of Myanmar pointed to targeted approaches as the only viable solution to achieving the goal. To help develop such targeted strategies, we have established a multidisciplinary team of research to bring together data from satellite earth observations, in situ surveys, and geospatial modeling, and conducted a study to assess the multi-scale exposure pathways that incorporate both landscape ecological settings and individual land use activities. At the landscape scale, forest cover strongly impacts village-level malaria prevalence, with the odds of a person having malaria increasing by a factor of 1.96 per 1 km^2 increase in natural forest cover within a 2 km radius of a village. Land use activities, specifically linked to natural forests and plantations, were identified as a major promoter of malaria acquisition. The data suggested that the higher the diversity of land use activities and their greater frequency and duration of land use, and the larger the individual's exposure to malaria transmission. In contrast, an individual's likelihood of contracting malaria dropped dramatically with other primary occupations (farming, student, or indoor worker). Our main conclusions point to the need for the new paradigm of intervention strategies to interrupt the exposure pathways outside of the home, in addition to existing home-centric approaches. Although these approaches were likely responsible for the progress in declining malaria burden, new tools and approaches are likely necessary for the last stretch towards final complete malaria elimination in Myanmar.

1410

FACTORS ASSOCIATED WITH CLUSTERING OF MALARIA CASES WITHIN THE INDEX CASE HOUSEHOLDS AND NEIGHBORHOOD HOUSEHOLDS IN ZANZIBAR

Abdul-wahid Al-mafazy[1], Abdullah Ali[2], Faiza Abbas[2], Mohamed Ali[2], Wahida Hassan[2], Makame Makame[2], Raya Ibrahim[2], Salum Massoud[2], Moza Khamis[2], Chonge Kitojo[3], Richard Reithinger[4], Mike McKay[4], Ssanyu Nyinondi[5], Joseph Joseph[5], Humphrey Mkali[5], Erik Reaves[6], Shabbir Lalji[5], Jeremiah Ngondi[7]

[1]*RTI International, Zanzibar, United Republic of Tanzania,* [2]*Zanzibar Malaria Elimination Programme, Zanzibar, United Republic of Tanzania,* [3]*U.S. President's Malaria Initiative/United States Agency for International Development, Dar es Salaam, United Republic of Tanzania, Dar-es-salaam, United Republic of Tanzania,* [4]*RTI International, Washington, DC, United States,* [5]*RTI International, Dar-es-salaam, United Republic of Tanzania,* [6]*U.S. President's Malaria Initiative, US Centers for Disease Control and Prevention, Dar es Salaam, United Republic of Tanzania, Dar-es-salaam, United Republic of Tanzania,* [7]*RTI International, Cambridge, United Kingdom*

Zanzibar introduced malaria case-based surveillance in 2012. District Malaria Surveillance Officers (DMSOs) investigate malaria cases notified by health facilities. Using a web-based surveillance system, DMSOs collect index case details at the diagnosing heath facility and household,

perform malaria rapid diagnostics tests (mRDT) for members of index case households, treat those with a positive mRDT result with antimalarial drugs, and complete case classification based on WHO guidelines. This data is transmitted to a central database for analysis and use. We aimed to evaluate the demographic characteristics, occupation, travel history, and use of preventive measures associated with malaria infection between July 2019 and February 2020. Logistical regression model was used to investigate factors associated with infection. Among the 9,181 index cases notified by health facilities, 5,064 (55%) were investigated to household level; 13,149 household members were tested by mRDT, of which, 426 (3%) were positive. Among the 5,490 total cases, 2,361 (43%) were classified as locally acquired and 3,129 (57%) as imported based on self-reported history of travel in the past 30 days. Of the 2,361 locally acquired cases, 158 (7%) had confirmed malaria infection in the past 30 days. Malaria infections among index cases and household members was associated with recent malaria infection in the past 30 days, OR 9.12, (95% CI 7.47-12.11), self-reported non-adherence to antimalarial drugs, OR 4.34 (95% CI 2.71-5.76), not using bednets, OR 1.11, (95% CI 1.04-1.19), and occupations as fishermen, OR 2.22 (95% CI 2.08-2.37) and police, OR 1.40 (95% CI 1.26 - 1.55). Reducing risk factors associated with infection might be addressed through social behavior change communication efforts to raise community compliance to treatment and use of preventive measures, such as bednets.

1412

TEMPORAL AND MICRO-SPATIAL HETEROGENEITY IN TRANSMISSION DYNAMICS OF CO-ENDEMIC *PLASMODIUM VIVAX* AND *P. FALCIPARUM* IN TWO RURAL COHORT POPULATIONS IN THE PERUVIAN AMAZON

Angel Rosas-Aguirre[1], Mitchel Guzman-Guzman[2], Raul Chuquiyauri[2], Marta Moreno[3], Paulo Manrique[4], Roberson Ramirez[4], Gabriel Carrasco-Escobar[2], Hugo Rodriguez[5], Niko Speybroeck[6], Jan Conn[7], Dionicia Gamboa[2], Joseph M. Vinetz[8], Alejandro Llanos-Cuentas[9]

[1]*Fund for Scientific Research FNRS; Research Institute of Health and Society (IRSS), Université catholique de Louvain, Brussels, Belgium,* [2]*Instituto de Medicina Tropical Alexander von Humboldt;Laboratorio ICEMR-Amazonia, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru,* [3]*London School of Hygiene & Tropical Medicine, Department of Immunology and Infection, London, United Kingdom,* [4]*Laboratorio ICEMR-Amazonia, Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru,* [5]*Dirección Regional de Salud Loreto DIRESA Loreto, Iquitos, Peru,* [6]*Research Institute of Health and Society (IRSS), Université catholique de Louvain, Brussels, Belgium,* [7]*Wadsworth Center, NYSDOH, Albany, NY, US; Department of Biomedical Sciences, School of Public Health, University at Albany, State University of New York, Albany, NY, United States,* [8]*Section of Infectious Diseases, Department of Internal Medicine, Yale School of Medicine, New Haven, CT, United States,* [9]*Instituto de Medicina Tropical Alexander von Humboldt; Facultad de Salud Pública y Administración, Universidad Peruana Cayetano Heredia, Lima, Peru*

Strategies that respond to highly heterogeneous and changing local malaria micro-epidemiology are needed to support control and elimination efforts. A 3-year, population-based cohort study was conducted in 2 different ecological settings in the Peruvian Amazon, Lupuna (riverine environment) and Cahuide (road-associated deforestation). 1,988 participants were rigorously followed by passive and active detection, allowing for accurate microscopically-confirmed malaria incidence rates (Jan 2013 and Dec 2015) and quarterly prevalence by quantitative real-time PCR. These measurements were related with environmental, entomological, genetic parasite diversity and routine surveillance/control data across time and space. LUP registered 1,708 *Plasmodium vivax* episodes in 627 participants and 120 *P. falciparum* episodes in 113 participants (80.7 total episodes/100person-years); while for CAH, 1,024 *P. vivax* episodes in 569 individuals and 20 *P. falciparum* episodes (40.2 episodes/100p-y). Following an increase in disease burden driven by floods and new *P. vivax* strains in LUP in early 2013, malaria incidence (mainly recurrent *P. vivax* episodes) and prevalence remained seasonally and at high levels despite control interventions and less favorable environmental conditions. Environmental-driven changes in vector behavior and human mobility in CAH triggered an epidemic transmission in 2012-2013, followed by significant reduction in incidence associated with persistent residual submicroscopic infections after outbreak responses in 2014 and early 2015, and a resurgence in late 2015. The proportion of participants with any *P. vivax* episodes was similar between LUP and CAH within the first year (~40%), differing by the end of the study (LUP:70·4%; CAH:51·4%). Intense and seasonal transmission resistant to standard control measures was observed in the malaria hyper-endemic LUP. Persistent low-level transmission continued in the malaria-vulnerable CAH after severe outbreaks were intensively handled. Control strategies failed in both settings to eliminate submicroscopic and hypnozoite reservoirs, enabling continued transmission.

1413

HIGH PROPORTION OF *PLASMODIUM VIVAX* RECURRENCES ARE DUE TO RELAPSE ACROSS DIVERSE GEOGRAPHICAL LOCATIONS

Robert J. Commons[1], Julie A. Simpson[2], James Watson[3], Nicholas J. White[3], Ric N. Price[1]

[1]*Menzies School of Health Research, Darwin, Australia,* [2]*Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, Australia,* [3]*Mahidol-Oxford Tropical Medicine Research Unit (MORU), Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand*

Infection with *Plasmodium vivax* can lead to dormant liver hypnozoites that can relapse causing symptomatic malaria weeks to months following initial infection. Relapsing infections cause substantial morbidity and are a source of transmission. To estimate the proportion of vivax malaria recurrences attributable to relapse we undertook a systematic review of clinical efficacy studies of uncomplicated vivax malaria where patients were treated with or without primaquine radical cure and were followed through multiple episodes for 365 days. The proportion of recurrences caused by relapses was estimated by assuming primaquine prevented all relapses and did not augment blood stage efficacy. From 261 studies identified, seven eligible studies were included, enrolling 4,256 patients from 15 treatment arm comparisons in eight countries. The overall pooled incidence rate ratio of vivax relapses for patients treated with primaquine compared with no primaquine was 0.15 (95%CI 0.10-0.21; I^2=81.9%); conservatively suggesting at least 79% of recurrences were caused by relapse. Pooled incidence rate ratios for countries varied from 0.05 in Pakistan to 0.34 in Nepal and Afghanistan. A high proportion of recurrent infections following schizontocidal treatment of acute vivax malaria are caused by relapse. Irrespective of location, effective hypnozoitocidal treatment of vivax malaria will substantially reduce morbidity and transmission from recurrent vivax malaria.

1414

CHARACTERIZING THE PHYSIOLOGY OF HEMOLYTIC TOXICITY IN A HUMANIZED MOUSE MODEL OF G6PD DEFICIENCY

Siobhan Flaherty[1], Pamela Strauch[1], Conner Jackson[1], Mahdi Maktabi[1], Larry Walker[2], Rosemary Rochford[1]

[1]*University of Colorado, Aurora, CO, United States,* [2]*University of Mississippi, Oxford, MS, United States*

Primaquine (PQ) is a clinically important radical cure for treatment of *P.vivax* infection, known to target hepatic hypnozoites. The substantial challenge with broad administration of PQ is the potential for hemolytic toxicity in individuals with glucose-6-phosphate dehydrogenase deficiency (G6PDd), yet the mechanisms of hemolytic toxicity remain unknown. To address this issue, we used a mouse model known to predict hemolytic toxicity responses in G6PDd red blood cells (RBC). NOD-SCID mice were

injected i.p. with G6PDd human (hu)RBC for 14 days, then treated with PQ at 15 mpk (a dose known to induce hemolytic toxicity) or vehicle. To evaluate markers of eryptosis, huRBC were isolated from mice 48 hrs post treatment and analyzed for phosphatidylserine (PS) on the cell surface and evidence of cell death using Annexin-V/PI staining. In addition, we measured levels of 2',7' -dichlorofluorescin diacetate (DCFDA) indicative of increased intracellular reactive oxygen species (ROS). Flow cytometry analysis of blood samples from PQ treated mice showed increased eryptosis as indicated by elevated PS and ROS levels as early as 48 hrs following treatment. This is coincident with an early rise in the levels of murine (mu) reticulocytes. To further investigate mechanisms of hemolytic toxicity, we evaluated spleen tissue harvested at 24 hrs and 7 days post treatment and histology was done to evaluate the presence of CD169+ marginal zone macrophages, F4/80+ red pulp macrophages, Ter119+ muRBC, and glycophorin A+ huRBC. Surprisingly, by 24 hrs following treatment, very few huRBC were detected in the spleen of PQ treated mice compared to vehicle control. By day 7, spleens of PQ treated mice were significantly enlarged compared to vehicle and had high numbers of muRBC, but a complete absence of any huRBC. Both macrophage populations were significantly depleted in PQ treated mice by 7 days. Further elucidation of the mechanisms of hemolytic toxicity using this model could provide much needed insight and help to inform and promote drug development.

1415

ANEMIA AND INFLAMMATION IN CHILDREN BELOW FIVE YEARS LIVING IN AREAS OF HIGH AND LOW TRANSMISSION SETTINGS IN WESTERN KENYA

Shehu S. Awandu[1], Lindsey B. Turnbull[2], Veronicah Knight[1], George Ayodo[1], John C. Chandy[2]

[1]*Kenya Medical Research Institute (KEMRI) / Jaramogi Oginga Odinga University of Science and Technology, Kisumu, Kenya,* [2]*Ryan White Center for Pediatric Infectious Disease & Global Health, Department of Pediatrics, Indiana University, Indianapolis, IN, United States*

Malaria may lead to anemia, but the extent to which anemia differs by malaria transmission rates in a community, and the relationship of inflammation in these areas to anemia is not well defined. We aimed to evaluate how anemia, parasitemia and inflammation differed in asymptomatic children <5 years of age in two communities, one with high, stable transmission and the other with low unstable transmission, and to assess how these factors related to each other within the communities. Among children <5 years of age, 24% (41/173) in the low malaria transmission area were anemic compared to 47% (91/192) in the high transmission area (p<0.001). Mean hemoglobin levels [SD] were correspondingly significantly higher in the low compared to high transmission area (11.8 [1.6] versus 10.9 [1.9] g/dL; p<0.001). C-reactive protein (CRP) concentrations were higher in the area of high compared to low transmission. Hemoglobin in the area of high, but not low, transmission was inversely correlated with inflammation (CRP concentration).10% (20/192) of children from the high transmission area and none of the children from the low transmission area had *P. falciparum* parasites by blood smear microscopy. In the high transmission area, hemoglobin levels were lower in malaria smear positive children compared to their smear negative counterparts (p=0.04). Inflammation in an area of high malaria transmission is associated with anemia, even in the absence of peripheral blood smear parasitemia. Further investigation is required to determine the sources of inflammation in children in the high malaria transmission setting (sub-microscopic or prior malaria vs. other infection vs. other source) and to evaluate how inflammation relates to iron deficiency, another common cause of anemia in low-income countries.

1416

SEVERE FALCIPARUM MALARIA IN YOUNG CHILDREN IS ASSOCIATED WITH POOR POST-DISCHARGE OUTCOMES: A PROSPECTIVE COHORT STUDY

Robert O. Opoka[1], Ruth Namazzi[1], Dibyadyuti Datta[2], Paul Bangirana[3], Andrea L. Conroy[2], Chandy C. John[2]

[1]*Makerere University, Kampala, Uganda,* [2]*Ryan White Center for Pediatric Infectious Diseases and Global Health, Indiana University School of Medicine, IN, United States,* [3]*Department of Psychiatry, College of Health Sciences, Kampala, Uganda*

Children treated for severe anemia in malaria-endemic areas are at high risk of post-discharge morbidity. However, post-discharge morbidity of children with common types of severe malaria (SM) have hitherto not been well described. We followed up a cohort of Ugandan children aged 6 months to 4 years for 12 months post-discharge who were admitted with one of the following forms of SM, as defined by the World Health Organization: Cerebral malaria (n=38), respiratory distress syndrome (n=75), multiple seizures (n=122), severe malarial anemia (n=85) and prostration (n=60). All clinical events that occurred during the follow up period were recorded. The rates of post-discharge readmissions or death were compared between children with SM and a cohort of community controls (CC, n=120) of similar age recruited from the families or neighborhood of the SM group and followed for a similar period of time. Overall, 212 (55.8%) children with SM had at least one readmission during the follow up period. Among the readmitted children, 97(45.8%) had multiple readmissions. Malaria was the major reason for readmission, accounting for 350 (83.3%) of the 420 readmission events. The rates of readmissions were similar across the SM groups (197 events per 100 person years, range 163-281 events per 100 person years among SM groups), and significantly higher than for CC, (64 events per 100 person years, P<0.001 compared to children with SM). Children with SM had a significantly higher risk of readmission or death compared to the CC group (HR=2.64, 95% CI 1.85-3.77). Similarly, the risk of readmission due to malaria was significantly higher in the SM group compared to CC (HR=2.81, 95% CI 1.89-4.18). Due to the increased risk of readmission and death among children with SM, there is an urgent need to provide post-discharge malarial chemoprophylaxis to all children treated for SM in malaria endemic areas.

1417

DIFFERENTIAL EXPRESSION OF UBIQUITYLATION PATHWAY GENES IN KENYAN CHILDREN WITH SEVERE MALARIAL ANEMIA

Samuel Bonuke Anyona[1], Evans Raballah[2], Qiuying Cheng[3], Ivy Hurwitz[3], Caroline Ndege[3], Elly Munde[3], Clinton Onyango[3], Nick Lauve[3], Kristan A. Schneider[4], Christophe G. Lambert[3], Benjamin H. McMahon[5], Collins Ouma[6], Douglas J. Perkins[3]

[1]*Maseno University School of Medicine, Kisumu, Kenya,* [2]*Masinde Muliro University of Science and Technology, Kakamega, Kenya,* [3]*Center for Global Health, Department of Internal Medicine, University of New Mexico, Albuquerque, NM, United States,* [4]*Department Applied Computer and Bio-Sciences, University of Applied Sciences Mittweida, Mittweida, Germany,* [5]*Theoretical Biology and Biophysics Group, Theoretical Division, Los Alamos National Laboratory, Los Alamos, NM, United States,* [6]*Maseno University School of Public Health and Comunity Development, Kisumu, Kenya*

In western Kenya, malaria remains one of the leading causes of childhood morbidity and mortality. The primary severe disease manifestation of *P. falciparum* infections in the holoendemic region is severe malarial anemia [SMA, hemoglobin (Hb)<5.0g/dL]. Our previous studies have shown that perturbations in inflammatory mediator production are associated with the pathogenesis of SMA. The effect of ubiquitylation on inflammatory mediators has been shown in other diseases, but the ubiquitin proteasome system (UPS) remains unexplored in human malaria. The UPS is the main cellular mechanism responsible for the degradation of intracellular

proteins in eukaryotic cells and is a key regulator of cellular processes. To establish the role of the UPS in malaria pathogenesis, we investigated transcriptional profiles of a set of 84 human ubiquitylation pathway genes in Kenyan children (n=44, aged <48 mos.) with either uncomplicated malaria (UM; Hb≥8.0g/dL; n=23) or SMA (Hb<6.0g/dL; n=21), recruited at Siaya County Referral Hospital in Western Kenya. Gene expression profiles were measured using the Human Ubiquitylation Pathway RT² Profiler PCR Array (Qiagen). Results showed that 16 out of 84 genes were dysregulated in children with SMA relative to UM. Of these, 10 genes were down-regulated and six genes were significantly up-regulated (P<0.050). Three genes displayed a magnitude ≥1.5 fold-change ($2^{-\Delta\Delta Ct}$): 1) up-regulation in SMA of Parkinson protein 2 (*PARK2*), which encodes for E3 ubiquitin protein ligase (P=0.015); 2) up-regulation in SMA of the gene encoding ubiquitin-conjugating enzyme E2M (*UBE2M, P*=0.032); and 3) down-regulation in SMA (P=0.015) of the gene for ubiquitin-conjugating enzyme E2D 1 *(UBE2D1*). Collectively, these results demonstrate differential expression of genes in the ubiquitylation pathway in children with SMA, supporting the notion that ubiquitylation may be involved in the pathogenesis of this disease.

1418

PERSISTENT DYSREGULATION OF METABOLISM IN CHILDREN WITH ACUTE MALARIA

Arlene E. Dent[1], Adam Pelletier[1], Leanne Robinson[2], Katherine Dobbs[1], Moses Laman[3], Rafick Sekaly[1], James Kazura[1]

[1]*Case Western Reserve University, Cleveland, OH, United States,* [2]*Burnett Institution, Melbourne, Australia,* [3]*PNG IMR, Madang, Papua New Guinea*

One goal of our research on malaria pathogenesis is to advance knowledge of how febrile malaria affects metabolite composition and how this impacts immune homeostasis. We collected plasma and peripheral blood mononuclear cells (PBMC) from Papua New Guinean 2-10 year old children at the time of presentation with acute febrile malaria (fever + *Plasmodium falciparum* (Pf) or *P. vivax* (Pv) monoinfection) and acute febrile non-malarial illness (*Plasmodium* spp. PCR-; e.g., pneumonia). Convalescent samples following antimalarial or antibiotic treatment were collected 9 weeks later. Matched acute and convalescent plasma from 25 children was analyzed for enriched metabolites by advanced mass spectrometry technology (Precision Metabolomics™). Differentially expressed metabolites (DEM, corrected for multiple comparisons) were selected. We adapted a Metabolite Set Enrichment Approach (MSEA) using a preranked list of metabolites which was then compared to SMPDB (Small Molecule Protein Database) and Metabolon subgroups. Comparing acute time points between malaria and acute non-malaria febrile illness, metabolic pathways involving diacylglycerol, dipeptides, sphingomyelins, ceramides, lysophospholipids, fatty acid metabolism acyl choline, glutathione and fibrinogen cleavage peptides were observed. However, a more striking contrast was observed when metabolites/pathways in convalescent plasma from malaria cases were compared to non-malaria cases. A total of 95 pathways differed for the SMPDB and Metabolon datasets combined (a greater number of differences than was observed among the acute sample comparisons). Many of the pathways involve amino acid, nucleotide, and lipid metabolism, but what was also featured were differences in energy metabolism. We interpret these results to indicate that children with acute malaria had persistent immune metabolic perturbations relative to children with non-malaria febrile illness who had more rapid recovery of metabolic homeostasis. Our ongoing studies will correlate these metabolomic data with PBMC immune cell gene expression.

1419

ACUTE KIDNEY INJURY AND PERSISTENT KIDNEY INJURY AT ONE MONTH FOLLOW-UP IN UGANDAN CHILDREN WITH SEVERE MALARIA

Andrea L. Conroy[1], Ruth Namazzi[2], Anthony Batte[2], Dibyadyuti Datta[1], John Ssenkusu[3], Robert O. Opoka[2], Chandy C. John[1]

[1]*Indiana University School of Medicine, Indianapolis, IN, United States,* [2]*Makerere University, Kampala, Uganda,* [3]*Makerere University School of Public Health, Kampala, Uganda*

Acute kidney injury (AKI) is increasingly recognized as an important clinical complication in children with severe malaria that is associated with increased mortality and is a risk factor for long-term neurocognitive and behavioral problems in surviving children. The objective of this study was to evaluate renal recovery in children admitted with severe malaria between 2014 and 2017 at Mulago National Referral Hospital in Kampala in Central Uganda and Jinja Regional Referral Hospital in Eastern Uganda. A total of 410 children with *P. falciparum* smear-positive severe malaria with an IDMS-traceable creatinine measure were included in the study. AKI on admission and acute kidney disease (AKD) at one-month follow-up were assessed using the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines. Baseline creatinine was estimated using the Pottel height-independent equation assuming a normal glomerular filtration rate (GFR) of 120mL/min per 1.73m^2 to back-calculate baseline creatinine. This equation has been validated in our setting to most closely estimate baseline creatinine in healthy community children with known creatinine. The prevalence of AKI in children with severe malaria on admission was 43.9% with 25.9% stage 1, 12.5% stage 2, 6.1% stage 3. AKI on admission was associated with an increased mortality with an adjusted odds ratio (aOR) of 8.42 (95% CI, 2.26 to 31.45) adjusting for age, sex, coma, acidotic breathing, severe anemia and site. AKI was more common in Jinja (55.6%) compared to Kampala (34.2%), p<0.0001. At one month follow-up, 38 children (11.9%) had evidence of persistent kidney disease with 95% of AKD observed in children from Jinja (p<0.0001). The presence of AKD at one-month follow-up was associated with 6.58-fold (95% CI, 0.94 to 46.04) increased odds of post-discharge mortality adjusting for age, sex, and site. Additional research is needed to understand how AKI impacts long-term kidney function and post-discharge morbidity and mortality in severe malaria survivors.

1420

IMPACT OF MALARIA ON FETAL GROWTH: A LONGITUDINAL ULTRASOUND COHORT STUDY IN BENIN

Valerie Briand[1], Emmanuel Yovo[2], Manfred Accrombessi[2], Auguste Degbe-Abagnon[2], William Atade[2], Titilola Ladikpo[2], Muriel Mehoba[2], Achille Massougbodji[2], Nicola Jackson[3], Nadine Fievet[4], Jennifer Zeitlin[5], François Koladjo[2]

[1]*Research Institute for Development (IRD), Bordeaux, France,* [2]*IRCB, Cotonou, Benin,* [3]*Oxford University, Oxford, United Kingdom,* [4]*Research Institute for Development (IRD), Paris, France,* [5]*Inserm, U1153, Paris, France*

There is a high level of evidence supporting the association between malaria in pregnancy and the risk of small-for-gestational age at birth. However, more evidence is required on the effect of malaria on fetal growth restriction (FGR). We assessed the effect of the timing of malaria during pregnancy on fetal growth using ultrasound data collected in Benin. We used data from the preconceptional RECIPAL cohort (2014-2017) conducted in southern Benin. Women were followed from the very first weeks of pregnancy until delivery; they benefited from 5 ultrasound scans (US). We assessed the effect of malaria on HC, BPD, AC and FL after adjustment for potential risk factors for FGR. Head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC) and femur length (FL) were transformed into z-scores using INTERGROWTH-21st charts. Both the timing of malaria and its effect over time were evaluated. Two different statistical models were used: a seemingly unrelated regression model to assess the effect of malaria on the 4 fetal parameters simultaneously, at

four time-points during pregnancy; a system of simultaneously equations regression model based on only one parameter to assess the effect of malaria on a given parameter throughout pregnancy. A total of 232 women with at least two US for fetal biometry and who gave birth to a live singleton were included in the analysis. Most of them had 5 US during follow-up at 7, 17, 22, 28 and 34 wg. The prevalence of malaria in the first, second and third trimesters was 21%, 16% and 13%, respectively. Both models yielded to similar results. We showed that malaria in the first trimester of pregnancy was significantly associated with an acute increase in all fetal parameters. This effect remained until the second trimester for BPD. Also, malaria in the third trimester was significantly associated with a decrease in all parameters at the end of the third trimester. In conclusion, we did not evidence a negative effect of malaria in the first trimester on fetal growth. However, malaria in the third trimester seemed to be associated with a decrease in all fetal parameters at the end of pregnancy.

1421

NEUREGULIN-1 ATTENUATES HEME- AND *PLASMODIUM FALCIPARUM* HISTIDINE RICH PROTEIN II - INDUCED INFLAMMATION AND NEURONAL DAMAGE IN CORTICAL BRAIN ORGANOIDS

Adriana Harbuzariu[1], Annette Nti[1], Juan Carlos Cespedes[1], Keri Harp[1], Andrew Shaw[2], Sha'aqua Asberry[2], Jonathan K. Stiles[1]

[1]*Morehouse School of Medicine, Atlanta, GA, United States,* [2]*Georgia Institute of Technology, Atlanta, GA, United States*

Human cerebral malaria (HCM) is a severe neurological manifestation of *Plasmodium falciparum* infection, which results in 20-30% mortality rates mainly in West African children. Our previous studies showed that erythrocyte hemolysis during parasite multiplication release heme and histidine rich protein II (HRPII) into circulation, causing blood brain barrier leakage, perivascular neuronal damage and placental disruption in placental malaria. HRPII antigen has been widely used in developing malaria diagnosis tests (RDT) and high plasma levels have been reported to correlate with HCM progression and severity. Previous models of HCM (2D cell culture and mouse experimental cerebral malaria) do not adequately recapitulate the direct effects of hemolysis and parasite-derived factors such as HRPII on brain development, inflammation, and neuronal injury. We utilized 3D cortical organoids to model HRPII-induced brain neuronal damage associated with HCM. We hypothesized that HRPII induces brain inflammation and reduces neuronal viability that can be attenuated by Neuregulin-1 (NRG-1), a neuroprotective factor. Flow cytometry, immunohistochemistry, western blot and ELISA/Multiplex immunoassay procedures were used to assess direct effects of HRPII on organoid growth, expression of markers of brain injury (BDNF, S100β, tau), and repair (NRG-1 and its receptor ErbB4). Neuronal apoptosis and brain organoid inflammation (CXCL10, Ang2/Ang1) in response to exposure to HRPII were assessed during organoid development. Our results show that exposure of brain organoids to physiologically relevant concentrations of HRPII during HCM compromised their structural architecture and altered expression of cleaved caspase 3 as well as key astrocyte, neuron, and microglia markers. Pro-inflammatory factors including CXCL10 and its receptor CXCR3, as well as ratio of Ang2/Ang1 were elevated. The HRPII effects were attenuated by NRG-1 via an ErbB4 mediated pathway. In conclusion, we utilized a brain organoid model to demonstrate that HRPII may play an important role in malaria-induced cortical damage and that NRG-1 attenuates the HRPII effects.

1422

QUANTIFYING AND PREVENTING *PLASMODIUM VIVAX* RECURRENCES IN PRIMAQUINE-UNTREATED PREGNANT WOMEN: AN OBSERVATIONAL AND MODELING STUDY IN BRAZIL

Rodrigo M. Corder[1], Antonio C. de Lima[1], David S. Khoury[2], Steffen S. Docken[2], Miles P. Davenport[2], Marcelo U. Ferreira[1]

[1]*University of Sao Paulo, Sao Paulo, Brazil,* [2]*University of New South Wales, Sidney, Australia*

Each year, 4.3 million pregnant women are exposed to malaria risk in Latin America and the Caribbean. *Plasmodium vivax* causes 76% of the regional malaria burden and appears to be less affected than *P. falciparum* by current elimination efforts. This is in part due to the parasite's ability to stay dormant in the liver and originate relapses within months after a single mosquito inoculation. Primaquine (PQ) is routinely combined with chloroquine (CQ) or other schizontocidal drugs to supress *P. vivax* relapses and reduce the risk of late blood-stage recrudescences of parasites with low-grade CQ resistance. However, PQ is contraindicated for pregnant women, who remain at increased risk of repeated infections following CQ-only treatment. Here we apply a mathematical model to time-to-recurrence data from Juruá Valley, Brazil's main malaria transmission hotspot, to quantify the extra burden of recrudescences attributable to PQ ineligibility in pregnant women. The model accounts for competing risks, since relapses and late recrudescences and new infections all contribute to recrudescences. We compare recurrence rates after primary *P. vivax* infections in 158 pregnant women treated with CQ only and after 316 *P. vivax* infections in non-pregnant control women, matched for age, date of infection, and place of residence, that were treated with a standard CQ-PQ combination. We estimated that, once infected with *P. vivax*, 23% of the pregnant women have one or more vivax malaria recurrences over the next 12 weeks; 86% of these early *P. vivax* recurrences were attributed to be likely relapses or late recrudescences, rather than new infections that could be prevented by reducing malaria exposure during pregnancy. Model simulations indicate that weekly CQ chemoprophylaxis extending over 4 to 12 weeks, starting after the first vivax malaria episode diagnosed in pregnancy, might reduce the risk of *P. vivax* recurrences over the next 12 months by 20% to 65%. We conclude that post-treatment CQ prophylaxis could be further explored as a measure to prevent vivax malaria recurrences in pregnancy and avert their adverse effects on maternal and neonatal health.

1423

MODELLING PUBLIC HEALTH IMPACT AND PRIMAQUINE OVERTREATMENT FOR SEROLOGICAL-TEST-AND-TREAT STRATEGIES TARGETING THE HIDDEN *PLASMODIUM VIVAX* RESERVOIR

Thomas Obadia, Michael White, Narimane Nekkab, Ivo Mueller

Institut Pasteur, Paris, France

Plasmodium vivax remains a major cause of malaria outside of Africa. Reaching pre-elimination conditions remains challenging because of the undetectable reservoir of liver-stage hypnozoites causing relapses that account for up to 80% of infections. The people most likely to carry hypnozoites can be identified using serological markers of exposures with 80% sensitivity and specificity, enabling Serological-Test-And-Treat (SeroTAT) approaches to target the hidden parasite reservoir while limiting exposure to 8-aminoquinolines and their potential for toxicity. Using the models from White et al., we estimated jointly public health impact and overtreatment achieved through SeroTAT strategies with varying diagnostic performance (sensitivity and specificity ranging 50-100%). The target population resembled that found in Papua New-Guinea subject to low or moderate *P. vivax* transmission pressures (qPCR prevalence resp. ~2% and ~10%). The impact of control strategies was measured as the reduction in *P. vivax* prevalence 6 months after intervention; overtreatment was defined as an administration of hypnozoiticidal drug that was unnecessary. Mass Drug Administration at 80% coverage with an ideal hypnozoiticidal

drug is predicted to cause a 66.3% reduction in *P. vivax* PCR prevalence. A single round of SeroTAT at 80% sensitivity and 80% specificity would lead to a 47.2%-56.4% reduction in prevalence, performing nearly as well as MDA with the benefit of a 3-10 fold reduction in overtreatment with hypnozoiticidal drugs. In all scenarios, public health impact of STAT was almost entirely dependent on the diagnostic test sensitivity. Increased specificity resulted in fewer people receiving unnecessary primaquine, with minor reductions in public health impact. SeroTAT is a viable alternative to MDA programs while limiting the risks inherent to 8-aminoquinolines. With such a targeted strategy, the benefits of reduced overtreatment allow for targeting the hidden *P. vivax* reservoir more safely.

1424

USING MECHANISTIC MODELS TO SUPPORT DECISION-MAKING IN COUNTRIES WITH HIGH MALARIA BURDEN

Monique Ambrose[1], Ifeoma D. Ozodiegwu[2], Aadrita Nandi[2], Kamaldeen Okuneye[2], Sebastian Rodiguez[2], Marita Zimmermann[1], Beatriz Galatas[3], Abdisalan Noor[3], Caitlin Bever[1], Jaline Gerardin[2]

[1]*Institute for Disease Modeling, Bellevue, WA, United States,* [2]*Northwestern University, Chicago, IL, United States,* [3]*World Health Organization, Geneva, Switzerland*

The National Malaria Control Programs (NMCPs) of countries with high malaria burden are frequently faced with difficult decisions about how to allocate limited malaria control resources. Many factors may influence the optimal use of interventions, including local malaria transmission intensity and seasonality, vector behavior, interventions used in the past, the cost of each intervention at different coverages, and the national budget. To help NMCPs estimate the expected impact of alternative intervention packages and identify the optimal strategy, we developed an analysis pipeline that uses data from a variety of sources, including climate data, national surveys, routine surveillance data, and intervention records, to parameterize a mechanistic model of malaria transmission dynamics and intervention effects. Working with NMCPs from high-burden countries, we combine bespoke models of malaria transmission in each of a country's health districts with an optimization framework to identify the intervention strategies expected to have the greatest impact on national malaria burden within operational and budget constraints. We compare the preferred strategies with alternative intervention mixes to quantify the value of the optimization approach and to highlight the benefits of subnational stratification.

1425

PROJECTED DEVELOPMENT OF ANTIMALARIAL DRUG RESISTANCE IN BURKINA FASO USING HIGH RESOLUTION SPATIAL MODELING

Robert J. Zupko[1], Thư Tran[1], Trần Đăng Nguyên[1], Fabrice Somé[2], Jean-Bosco Ouédraogo[2], Maciej F. Boni[1]

[1]*Pennsylvania State University, State College, PA, United States,* [2]*Institut de Recherche en Sciences de la Santé, Direction Régionale de l'Ouest, Bobo-Dioulasso, Burkina Faso*

P. falciparum malaria is holoendemic in Burkina Faso and accounts for about 61.5% of hospitalizations and 30.5% of deaths. Artemether-Lumefantrine (AL) along with Artesunate-Amodiaquine (AS-AQ) combination therapy have been in use as first-line therapies for uncomplicated malaria since 2005, contributing to a 32.1% reduction of the incidence in malaria from 2000 to 2017. However, the use of artemisinin combination therapies (ACTs) potentially puts the country at risk if drug resistance develops in *P. falciparum*. To investigate how drug resistance may manifest in Burkina Faso, we have improved upon a previously developed individually-based stochastic mathematical model to better account for the geographic distribution of the population along with the short to medium-term migration of individuals. These improvements allow for projections to be made at the 25 sq.km level throughout the country, resulting in about 11,000 grid points mapped to the country's 45 provinces. The high-resolution nature these improvements contributes to drug resistance surveillance efforts and offers the possibility for National Malaria Control Programs to project the impacts of localized interventions. We show under which conditions drug-resistance emerges more and less quickly in a high-transmission malaria setting comparable to Burkina Faso.

1426

USING MALARIA ANTIGEN DATA AND MACHINE LEARNING MODELS TO CLASSIFY MALARIA INFECTIONS AND STRATIFY VILLAGES BY PREVALENCE LEVEL

Leo Zoeckler[1], **Hannah Slater**[1], Ihn Kyung Jang[1], Allison Golden[1], Francois Nosten[2], Jordi Landier[3], Gonzalo J. Domingo[1]

[1]*PATH, Seattle, WA, United States,* [2]*Mahidol Oxford Tropical Medicine Research Unit, Shoklo Malaria Research Unit, Bangkok, Thailand,* [3]*French National Research Institute for Sustainable Development, Marseille, France*

Innovative approaches must be taken for malaria-endemic countries with low transmission to achieve elimination. PCR is a commonly used tool in research settings to measure prevalence in areas approaching elimination, but it is a time-intensive, nonstandard process that requires access to a well-equipped laboratory and trained technicians—not feasible in many elimination settings. We propose as an alternative, the Quansys Biosciences Q-Plex™ Human Malaria Array (4-plex), which quantifies three malaria antigens and a human inflammatory biomarker. Coupled with a machine learning (ML) model, we leveraged data generated using the Q-Plex assay to predict the infection status and parasite species of blood samples and compared results against PCR-confirmed data. We used data from a study of 1,808 samples from individuals in 22 villages in a low-transmission area in Myanmar. Each sample contained data on three malaria antigens, a human inflammatory biomarker, and a binary outcome (PCR infection status). We then used TPOT, an autoML tool, to search through a large set of potential algorithms, data transformations, and different combinations therein, and generate a best model. This model is used to classify samples in terms of their infection status (positive or negative). The best model, a stacked stochastic gradient descent classifier/extra-trees classifier, performed better than existing RDTs and single-antigen logistic regression models and had a sensitivity of 98% and a specificity of 90% compared to PCR. Furthermore, we developed a "proof of principle" algorithm that uses the probability of positivity predictions from the model to stratify villages based on prevalence. Initial predictions indicate the ML approach drastically outperforms RDTs in stratifying villages into high and low prevalence. As more areas approach elimination, researchers and policymakers need access to an effective, comparatively noninvasive method for estimating prevalence in low-transmission settings. The Q-Plex and similar assays, in combination with a robust ML model, present a novel, promising alternative to both PCR and RDT within this context.

1427

TRANSLATING OBSERVATIONAL STUDIES FOR DISEASE MODELLING

Theresa Reiker[1], Thomas Smith[1], Manuela Runge[2], Melissa Penny[1]

[1]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [2]*Northwestern University, Chicago, IL, United States*

Realistically representing natural settings in disease modelling is required for a multitude of applications. This includes generating site-specific predictions and recommendations on optimal intervention strategies, the exploration of retrospective *what-if* or counter factual experiments, and model calibration. However, this process presents great challenges, as it requires having a significant understanding of a setting's characteristics. This includes information on demography, efficiency of the local health care system and the population's care seeking behaviour, history of disease control interventions, possible seasonal patterns, and disease-specific aspects, such as the abundance of different vector species and their setting-specific behaviours like biting patterns. Without expert knowledge of the setting relevant information on a range of topics must be collated

from databases, the published literature (which may include publications in highly specialised journals and or in multiple languages), epidemiological and demographic surveys reports, and other sources. As a result, the problem is often circumvented rather than solved through conduction of experiments in hypothetical, archetypical or generic settings. Here, we present a framework for formalizing and translating epidemiological studies on the prevalence-incidence relationship of *Plasmodium falciparum* for simulation in individual-based models. The studies are replicated in an established individual-based stochastic simulator *OpenMalaria*. Our simulation results illustrate that capturing the complexity of a field site is important to making reasonable predictions and that it is insufficient to account for transmission intensity and seasonality alone. It follows that the applicability of predictions depends heavily on researchers taking appropriate measures to accurately represent studies in all their complexity.

1429

IMPACT OF SEASONAL MALARIA CHEMOPREVENTION BEYOND MALARIA CONTROL AMONGST UNDER-FIVE CHILDREN IN EIGHT LOCAL GOVERNMENT AREAS ACROSS KATSINA AND YOBE STATES.

Akinola Shonde[1], Frank Oronsaye[1], Jamilu Nikau[2], Olatayo Abikoye[1], Jane Onyilo[3], Nnenna Ogbulafor[2], Yakubu Cherima[1], Bala Mohammed[2], Diwe Ekweremadu[1], Sonachi Ezeiru[1], Olugbenga Mokuolu[2]

[1]*Catholic Relief Services Nigeria, Abuja, Nigeria,* [2]*National Malaria Elimination Program, Abuja, Nigeria,* [3]*Malaria Consortium, Abuja, Nigeria*

Seasonal Malaria Chemoprevention (SMC) is aimed at reducing malaria-related morbidity & mortality amongst under 5 children during the period of greatest risk in the Sahel sub-region. It involves cyclical administration of Sulphadoxine-Pyrimethamine & Amodiaquine by Community Health Volunteers, CHVs during the peak of rainy season. Several studies have shown the impact of SMC on malaria control. However, beyond malaria, this study explores the impact of SMC on; number of identified monthly morbidity cases in the community by the CHVs, and; all-cause mortality, amongst under 5 children during the 4 cycles of 2019 SMC implementation in 8 intervention Local Government Areas (LGAs) across the 2 states. A cross-sectional analysis of 2019 SMC program data (cycle1-4) in 4 intervention LGAs each in Katsina State (Daura, Jibia, Kaita, Zango) & Yobe State (Bade, Karasuwa, Machina, Nguru) shows a downward trend in the number of morbidity cases referred to the HFs from the 1st cycle (July) to the 4th cycle (October) of SMC implementation, despite no significant reduction in the total number of CHVs participating in each cycle, and all other relevant factors were observed to be the same throughout the implementation period across the LGAs. There is a 70% (2891 to 857 cases in Katsina) & 78% (696 to 156 cases in Yobe) reduction in total number of cases referred to the HF from the 1st to the 4th cycles (July - October 2019 respectively) in the 8 LGAs across the 2 states. Also, secondary data on all-cause mortality on District Health Information System 2, DHIS-2 shows a 45% (in Yobe) & 82% (in Katsina) decline in total number of < 5 mortality from 2018 (no SMC) - 2019 (post-SMC) in the 8 intervention LGAs across the 2 states. These observations can be correlated to SMC processes including mass drug administration & referral. It is evident that the impact of SMC extends beyond just malaria control, as other identified sick under 5 children are referred to the health facility for care, thereby improving general morbidity outcomes in communities. SMC was demonstrated to show these added impacts. Implications & opportunities for the health system, and value chain analysis discussed.

1430

ASSESSING THE IMPACT OF SEASONAL MALARIA CHEMOPREVENTION ON SUSPECTED AND CONFIRMED MALARIA CASES IN 10 HEALTH DISTRICTS IN CHAD USING ROUTINE CLINICAL DATA, 2013-2018

Sol Richardson[1], Azoukalne Moukenet[2], Arantxa Roca-Feltrer[1], Monica A. de Cola[1], Zana Coulibaly[2]

[1]*Malaria Consortium, London, United Kingdom,* [2]*Malaria Consortium, N'Djamena, Chad*

The World Health Organization recommends prophylactic administration of sulfadoxine-pyrimethamine plus amodiaquine to children aged 3-59 months at monthly intervals, as seasonal malaria chemoprevention (SMC), against *Plasmodium falciparum* malaria in areas where transmission is highly seasonal. Although SMC has been found efficacious in clinical trials, reducing confirmed cases by around 75%, there remains a need to generate evidence of impact at scale using different data sources. Using routine clinical data reported through the national Health Management Information System from 2013-2018, we analysed associations between SMC administration during the high-transmission season (July-October) and monthly district-level counts of suspected and confirmed malaria cases among children aged 0-59 months in 10 health districts with available estimates of catchment population. Generalized additive models with a negative binomial distribution were fitted with separate cyclic cubic spline terms for each district to adjust for seasonality in cases. Models were also adjusted for prevalence of malnutrition (determined using the weight-for-height method) and use of mosquito nets among children aged 0-59 months at the region level, obtained from the 2014 Chad Demographic and Health Survey. Offset terms corrected for differences in district-level population and projected population growth. SMC administration was significantly associated with lower adjusted monthly counts of both suspected (incidence rate ratio [IRR]: 0.75, 95% CI: 0.67-0.84, p<0.001) and confirmed cases (IRR: 0.73, 95% CI: 0.65-0.82, p<0.001). Prevalence of mosquito net use was associated with lower counts of suspected cases. The model results, based on routine data, support effectiveness of SMC at scale and show a reduction of 25% in malaria incidence. Further work will investigate determinants of program effectiveness at scale and extend the analysis to include 43 eligible districts and a wider range of data sources (e.g. climatic variables) while refining offset terms to correct for non-reporting at the clinic level using estimates of catchment populations.

1431

EXTENDING SEASONAL MALARIA CHEMOPREVENTION IN BURKINA FASO TO FIVE CYCLES TO COINCIDE WITH THE START OF THE RAINY SEASON IN THE CASCADES REGION: RESULTS FROM A PILOT STUDY TO ASSESS FEASIBILITY, ACCEPTABILITY, COST AND IMPACT ON MALARIA INCIDENCE

Kevin Nicholas Baker[1], **Adama Traore**[2], Charlotte Ward[1], Benoit Sawadogo[2], Helen Counihan[1], Christian Rassi[1], Sol Richardson[1], Helen Smith[1], Johanna Johansson[2], Justin Savadogo[3], Alain Toe[2]

[1]*Malaria Consortium, London, United Kingdom,* [2]*Malaria Consortium, Ouagadougou, Burkina Faso,* [3]*National Malaria Control Programme, Ouagadougou, Burkina Faso*

Across the Sahel, the majority of malaria illness and deaths in children under five years occur during the rainy season. In 2012, the World Health Organization (WHO) issued a policy recommendation for seasonal malaria chemoprevention (SMC), the administration of antimalarial drugs at monthly intervals during this high transmission period, usually four months, to protect children 3-59 months of age in this region. While SMC is implemented from July to October every year in Burkina Faso, in parts the rainy season now starts earlier. In June 2019, Malaria Consortium implemented an additional monthly SMC cycle in Mangodara health district in the south, where this early start has been observed. A mixed-methods pilot implementation research study assessed if this extension is feasible and acceptable, and its impact on malaria incidence in children 3-59 months. Qualitative data from eight focus group discussions with

caregivers and community distributors and 11 key informant interviews with community, program and national level stakeholders were collected and analyzed. Coverage data were collected using an end of round household survey. Quantitative results show 87.7% (95% CI 85.6-89.5) of day 1 treatments were administered during the additional cycle, compared to 87.2% (95% CI 83.4-90.3) for another 23 health districts in July 2019. Preliminary qualitative findings suggest caregivers, community distributors and supervisors believed that a reduction in malaria cases was associated with the earlier campaign. However, some concerns were raised, including the earlier cycle coinciding with agricultural work; difficulty accessing certain areas owing to flooding in June; remuneration issues; and lack of understanding among caregivers about why SMC started earlier. Key informants at national and regional level suggested need for more evidence to demonstrate that five SMC cycles has additional impact on reducing under-five mortality. Results suggest if the campaign starts earlier, similar coverage can be expected in the first cycle compared to health districts starting in July and that the health benefits were recognized widely.

1432

MEASURING IMPACT OF SEASONAL MALARIA CHEMOPREVENTION IN BURKINA FASO USING NATIONAL HOUSEHOLD SURVEYS (2010-2017)

Monica Anna deCola[1], Benoit Sawadogo[2], Sol Richardson[1], Arantxa Roca-Feltrer[1], Christian Rassi[1]

[1]*Malaria Consortium, London, United Kingdom,* [2]*Malaria Consortium, Ouagadougou, Burkina Faso*

Seasonal Malaria Chemoprevention (SMC) has shown to have a 75% protective efficacy against malaria episodes as measured from 7 clinical trials. It can be expected that impact under programmatic conditions will differ from impact determined in experimental settings. To identify reliable and sustainable methods of measuring impact at scale using routine data, estimates generated need to be verified and compared with other data sources and internal and external factors affecting impact need to be assessed. Data from population based household surveys (Demographic Health Surveys from 2010 and Malaria Indicator Surveys from 2014 and 2017) in Burkina Faso were overlaid with SMC programme data, geographic data from the Burkina Faso Mapping Institute, and rainfall data from the Burkina Faso Meteorological Institute to asses impact of SMC on malaria and anaemia (indirect). Data were first analysed at the district level to understand population level factors and adjustments needed at the individual level for impact. Point prevalence of malaria in 6-59 month olds as diagnosed by rapid diagnostic test (RDT) in 2010 before SMC was 76% (95%CI 75-78%). In 2014, 7 districts introduced SMC and had a mean prevalence of 43% (CI 40-46%). Sixty-two districts did not have SMC and had a mean prevalence of 67% (CI 62-72%). In 2014, there was a significant drop in prevalence across all districts, this was most likely due to survey timing after the rainy season. A mixed effects logistic regression with random intercepts for district was conducted to estimate the level of impact of SMC. A cubic spline was fitted to adjust for seasonal factors other than rainfall. Preliminary results indicate there is strong evidence that after controlling for survey, month, rainfall, age, urbanisation, and net use that there is a decrease in odds of RDT confirmed malaria in districts with SMC (OR 0.38, 95% CI: 0.32 – 0.45, p<0.001). Results were similar for microscopy. SMC has also shown a protective effect for anemia (OR 0.72, 95% CI: 0.60 – 0.88, p<0.001). These findings suggest that SMC is having a significant impact on prevalence and standard survey data can be used to monitor impact as well as complement analyses using routine data.

1433

DIFFERENTIAL REDUCTION IN FACILITY-LEVEL AND DISTRICT-LEVEL MALARIA CASES FOLLOWING A NATIONAL MASS BEDNET DISTRIBUTION CAMPAIGN WITH TWO TYPES OF NETS, MALAWI, 2018-2019

Collins Kwizombe[1], Tyson Volkmann[1], Kevin Griffith[2], Julie Gutman[3], Pius Masache[1], Lilia Gerberg[2], John Gimnig[3], Austin Gumbo[4], Michael Kayange[4], Edson Dembo[1], Monica Bautista[1], John Painter[3]

[1]*PMI, Lilongwe, Malawi,* [2]*PMI, Washington, DC, United States,* [3]*CDC, Atlanta, GA, United States,* [4]*NMCP, Lilongwe, Malawi*

In recent years, resistance to pyrethroid insecticides has increased among the main malaria vectors in Malawi. Piperonyl butoxide (PBO) long-lasting insecticide-treated nets (LLINs) may counteract pyrethroid resistance, but their potential impact in Malawi is unknown. Recognizing the need to scale up and monitor new tools to prevent resistance and preserve gains made in malaria control, Malawi's National Malaria Control Programme implemented a pilot of PBO LLINs in 10 districts during a mass net distribution campaign from September to November 2018. Standard pyrethroid LLINs were distributed in the remaining 18 non-indoor residual sprayed districts in 2018. To measure the difference in the number of malaria cases before and after the intervention, we conducted a time-series analysis using routine surveillance data merged with reported monthly stockout days, outpatient department attendance, and precipitation data. Confirmed malaria cases from 381 of the 787 facilities reporting into a District Health Information System 2 database from January 2015-2018 were used to build a predictive autoregressive integrated moving average model and a counterfactual, controlling for stockout and precipitation covariates, to compare the decrease in actual cases in PBO LLIN districts versus standard LLIN districts during 2019. We also compared the changes in crude reported cases at the district level in 2019 by net type distributed. In the adjusted model, facilities in districts with PBO LLINs had a significant mean decline of 35% compared with a decline of 21% for facilities in standard LLIN districts from 2018 to 2019. At the district level, overall cases significantly declined by 53% in PBO LLIN district versus 16% in standard LLIN. These results suggest that PBO nets were associated with higher relative reductions in malaria cases. Furthermore, the difference in impact was detected through routine health data, highlighting the importance of these data for decision making. New net types are a critical tool that Malawi must utilize to achieve malaria control. Further monitoring of these nets and the expected declines in incidence is warranted.

1434

GROUP ANTENATAL CARE: A BASELINE INITIATIVE TO IMPROVE MALARIA IN PREGNANCY AND ANTENATAL CARE ATTENDANCE INDICATORS. A CASE FROM GEITA TANZANIA

Jasmine Chadewa[1], Mary Drake[1], Chonge Kitojo[2], Ryan Lash[3], Stephanie Suhowatsky[4], Abdalah Lusasi[5], Japhet Simeo[6], Goodluck Tesha[7], Ruth Lemwayi[1], Issa Garimo[5], Agnes Kosia[1], Alice Christensen[1], Rita Noronha[1], Zahra Mkomwa[7], Naomi Serbantez[2], Melkior Assenga[1], Erik Reaves[8], Samwel Lazaro[5], Miriam Kombe[9], Alen Kinyina[1], Alen Kinyina[1], Ally Mohamed[5], Gladys Tetteh[4], Bill Brieger[10], Edward Kenyi[4], Annette Almeida[1], Julie Gutman[3]

[1]*USAID Boresha Afya Project -Jhpiego Tanzania, Dar es Salaam, United Republic of Tanzania,* [2]*President's Malaria Initiative/United States Agency for International Development, Tanzania, Dar es Salaam, United Republic of Tanzania,* [3]*Malaria Branch, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Dar es Salaam, United Republic of Tanzania,* [4]*Jhpiego Headquarter U.S.A, Baltimore, MD, United States,* [5]*National Malaria Control Program-Tanzania Ministry of Health, Community Development, Gender, Elderly and Children, Dar es Salaam, United Republic of Tanzania,* [6]*Regional Health Management Team, Dar es Salaam, United Republic of Tanzania,* [7]*USAID Boresha Afya Project –Path Tanzania, Dar es Salaam, United Republic of*

Tanzania, [8]President's Malaria Initiative, Centers for Disease Control and Prevention Tanzania, Dar es Salaam, United Republic of Tanzania, [9]USAID, Dares Salaam, Dar es Salaam, United Republic of Tanzania, [10]Jhpiego Headquarter U.S.A, Dar es Salaam, United Republic of Tanzania

Malaria in pregnancy (MiP) is a major public health concern contributing to poor maternal and newborn health outcomes. Early and frequent Antenatal Care attendance (ANC) could address this problem. Early ANC booking is still low in Tanzania. USAID Boresha Afya and Tanzania Ministry of Health introduced a Group Antenatal Care (GANC) initiative in Geita region where malaria prevalence is high. This model brings 8-15 pregnant women of similar gestational age together for ANC. Group contacts last 1.5-2 hours, and include clinical care, information sharing, and peer support to improve quality of care and women's engagement, leading to better retention in care. Prior to implementation, a baseline cross-sectional household survey was conducted in December 2019 in 40 communities across Geita region. The survey was intended to identify gaps and targets in MiP services delivery which could be addressed through GANC. Women who had delivered a live born infant in the preceding 12 months were included. We interviewed 1111 women; mean age was 27 years. One-third had no education and only 9% had secondary education. Nearly all 95% of women lived in a house with an Insecticide Treated Net (ITN); 87% reported receiving an ITN during their last pregnancy and 90% reported ITN use on the night before the survey. Nearly all 98% attended ANC at least once, with 17% attending in first trimester. Only 45% attended ≥4 visits; 6% of women were stopped by their husbands from attending ANC. Median total time spent away from the home for each ANC was 4 hours. 88% received Intermittent Preventive Treatment of malaria in pregnancy (IPTp), with 53% receiving the recommended 3 or more doses. Among those who did not receive IPTp, 42% reported that the provider did not offer it and 25% reported it was not available at the facility. Receipt of other interventions varied: 64% reported that their blood pressure was checked, 95% and 57% had blood and urine samples collected, 74% had received adequate doses of tetanus vaccination, and 94% received iron/folate supplements. We will assess whether GANC improve MiP services as well as quality of ANC care, to promote positive pregnancy outcomes.

1435

MODELING NOVEL GENETIC CONTROL STRATEGIES FOR *AEDES AEGYPTI* DISEASE VECTORS

John M. Marshall[1], Héctor M. Sánchez C.[1], Jared B. Bennett[1], Sean L. Wu[1], Tomás M. León[1], Gordana Rašić[2], Omar S. Akbari[3]

[1]*University of California, Berkeley, Berkeley, CA, United States,* [2]*QIMR Berghofer Medical Research Institute, Brisbane, Australia,* [3]*University of California, San Diego, La Jolla, CA, United States*

The discovery of CRISPR-based gene editing, followed in quick succession by the 2015 Zika epidemic in Latin America and the Caribbean, led to substantial interest in the development of novel genetics-based strategies to control *Aedes aegypti* mosquitoes and the diseases they transmit. Vector control is of particular interest in the absence of therapeutic drugs or an effective vaccine for dengue, Zika and chikungunya viruses. The use of homing-based gene drive systems has been greeted with excitement, for the potential to control mosquito-borne diseases on a wide scale, and concern, for the invasiveness and potential irreversibility of a release. Threshold-dependent gene drive systems, such as reciprocal chromosomal translocations, have been proposed as an alternative during the trial phase of this technology, or when localized control is otherwise desired, as simple models predict them to spread into partially isolated populations in a confineable manner and to be eliminated through dilution with wild-type organisms. Other self-limiting gene drive systems display transient spread, such as split drive and daisy drive, before being eliminated by virtue of a fitness cost. Additionally, CRISPR-based versions of the sterile insect technique provide a means to suppress wild populations of mosquitoes through large-scale releases of eggs into the environment. We discuss the potential role that each of these technologies could play in controlling *Ae. aegypti* populations through simulating their release in spatially-explicit environments. Simulations are implemented using the Mosquito Gene Drive Explorer (MGDrivE) modeling framework, with genetics-based strategies parameterized using laboratory-generated data, and mosquito population dynamics parameterized using data from mark-release-recapture experiments and genomic data. Results confirm the confineability of threshold-dependent and self-limiting systems, and their potential role in a phased introduction of genetic technologies for the control of mosquito-borne diseases.

1436

OPPORTUNISTIC BLOOD HOST UTILIZATION AND SPATIAL HETEROGENEITY OF *ANOPHELES* BITES PROMOTE PERSISTENT MALARIA TRANSMISSION IN MADANG, PAPUA NEW GUINEA

John B. Keven[1], Michelle Katusele[2], Rebecca Vinit[2], Daniella Rodriguez-Rodriguez[3], Manuel Hetzel[3], Moses Laman[2], Leanne Robinson[4], Stephan Karl[5], Edward D. Walker[1]

[1]*Michigan State University, East Lansing, MI, United States,* [2]*PNG IMR, Madang, Papua New Guinea,* [3]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [4]*Burnet Institute, Melbourne, Australia,* [5]*Australian Institute of Tropical Health and Medicine, Townsville, Australia*

The ongoing nationwide distribution of long-lasting insecticidal nets (LLIN) against *Anopheles* vectors of malaria since 2008 resulted in reduction of infection prevalence and transmission rate of malaria in coastal villages in Madang, Papua New Guinea. However, recent data show that transmission rate has persisted between 10-160 infective bites per person-year since 2011, and prevalence of infection rebounding from an average of 9% to 18% between 2014-2017 for *Plasmodium falciparum* and 13% to 20% between 2010-2014 for *Plasmodium vivax*, despite high bednet use in this region. The present study investigated factors sustaining persistent transmission, including blood host utilization and heterogeneity of human exposure to infectious bites from local vectors in four villages. Blood-fed mosquitoes were sampled using barrier screens and blood-meal hosts were identified by PCR. Human blood meals were fingerprinted by microsatellite genotyping to identify the individuals bitten. Vector biting rate per person-night was assessed by human-landing catches inside and outside houses in different locations within villages. The local vectors were opportunistic in their host utilization and fed on both humans and non-humans rather than strictly anthropophilic. The frequency of blood meals from different individual humans was not random but rather clustered, and a few individual villagers were utilized as hosts more frequently than most others. The biting frequency was clustered spatially and was high in few locations and low in most locations. Basic reproduction rate (R_0) of malaria estimated from the spatial data ranged from 1.7-4.6. Most (50%) of the bites occurred outdoors rather than indoors and in the evening before 10 PM (29-50% of total) rather than later in the night. The opportunistic host utilization and spatial clustering of outdoor biting behavior of the local vectors enable them to evade LLIN by feeding on alternative hosts and transmit malaria by feeding on humans outdoors and early in the evening, when people are active and unprotected by LLINs. Estimates of R_0 exceeding 1.0 enable malaria transmission to persist.

1437

THE *ANOPHELES GAMBIAE* VITELLOGENIN REGULATES FERTILITY AND AFFECTS *PLASMODIUM FALCIPARUM* DEVELOPMENT

Iryna Stryapunina, Lydia Mendoza, Maurice A. Itoe, W. Robert Shaw, Kristine Werling, Flaminia Catteruccia

Harvard University, Boston, MA, United States

The female *Anopheles gambiae* mosquito feeds on human blood for egg development. After ingesting a bloodmeal females produce a yolk precursor protein called vitellogenin (Vg) and deposit it into the oocyte by receptor-mediated endocytosis for amino acid needs of the embryo. Recently, we have identified that *Vg* RNAi knock-down in *An. gambiae* females causes almost complete infertility. Vg-depleted females mate normally but develop significantly fewer eggs. We determined by

immunofluorescence microscopy that Vg-depleted eggs are devoid of yolk crystals and show significant accumulation of lipids. Upon laying, the eggs do not melanize, and although they are fertilized they do not hatch. Furthermore, we analyzed the role of Vg in the development of the human malaria parasite *Plasmodium falciparum*, whose initial phases of development temporally coincide with Vg incorporation into the eggs. Our data show that Vg depletion does not affect parasite numbers but increases their growth rates, a phenotype not detected in the mouse malaria model *P. berghei*. We are currently elucidating the specific mosquito-parasite interactions affected by the function of this nutrient transporter. Overall, the observed phenotypes following Vg depletion not only pose interesting basic biological questions of the role of this transporter in mosquito fertility and parasite development but may also highlight novel opportunities for vector control.

1438

RELEVANCE OF ENTOMOLOGICAL MONITORING DATA IN DECISION MAKING FOR APPROPRIATE AND SUSTAINABLE MALARIA VECTOR CONTROL IN CÔTE D'IVOIRE

Bernard L. Kouassi[1], Constant V. Edi[2], Emmanuel Tia[3], Lucien Y. Konan[4], Maurice A. Akré[5], Alphonsine A. Koffi[5], Allassane F. Ouattara[2], Antoine Tanoh Mea[6], Constant G. Gbalegba[1], Pascal Zinzindohoue[7], Blaise Kouadio[7], McKenzie Andre[7], Seth R. Irish[8], Jennifer Armistead[9], Dereje Dengela[10], Ndombour G. Cissé[1], Cecilia Flatley[10], Joseph Chabi[1]

[1]*PMI VectorLink Project, Abidjan, Côte D'Ivoire,* [2]*Swiss Center of Scientific Research, Abidjan, Côte D'Ivoire,* [3]*Centre of Veterinary and Medical Entomology, Abidjan, Côte D'Ivoire,* [4]*National Institute of Public Hygiene, Abidjan, Côte D'Ivoire,* [5]*National Institute of Public Health/ Pierre Richet Institute, Bouaké, Côte D'Ivoire,* [6]*National Malaria Control Programme, Abidjan, Côte d'Ivoire, Abidjan, Côte D'Ivoire,* [7]*U.S. President's Malaria Initiative, USAID, Abidjan, Côte D'Ivoire,* [8]*U.S. President's Malaria Initiative, Entomology Branch, U.S. Centers for Disease Control and Prevention, Atlanta, GA, United States,* [9]*U.S. President's Malaria Initiative, USAID, Washington, DC, United States,* [10]*PMI VectorLink Project, Washington, DC, United States*

Insecticide susceptibility, resistance intensity and piperonyl butoxide (PBO) synergist assays were conducted using WHO susceptibility tube tests and CDC bottle assays on *An. gambiae* s.l. from 15 sites in Côte d'Ivoire. In addition, monthly vector bionomics were conducted in four sites with particularly high malaria incidence: Sakassou from January to December 2019 and Beoumi, Dabakala and Nassian from May to December 2019. Human landing catches (HLCs), pyrethrum spray catches (PSCs) and United States Centers for Disease Control and Prevention (CDC) light traps were used to conduct longitudinal vector behavior monitoring. High pyrethroid resistance was observed in all 15 sites. Pre-exposure to PBO before pyrethroids yielded a substantial increase in the mortality of *An. gambiae* s.l. particularly with deltamethrin. Chlorfenapyr (200µg/bottle) yielded susceptibility in 10 of the sites. Susceptibility to clothianidin and pirimiphos-methyl was observed in 7 and 10 sites, respectively. *Anopheles gambiae* s.l. was the predominant malaria vector collected in all four bionomics sites. Vector density was relatively high in Sakassou throughout the year with mean biting rates of 230 bites per person per night (b/p/n) and an entomological inoculation rate (EIR) of 7.8 infective bites per person per night (ib/p/n)). In Beoumi, Dabakala and Nassian, vector densities were lower with mean biting peaks of 50.4 b/p/n. The highest EIRs were recorded in October for Beoumi (0.8 ib/p/n), in July for Dabakala (2.3 ib/p/n), and in October for Nassian (1.6 ib/p/n). Based on these results, the NMCP has developed a stratified mass distribution plan for next generation ITNs in 2021 to manage the high pyrethroid resistance observed. These results also supported the selection of clothianidin products for the 2020 IRS campaign in two high-burden districts (Sakassou and Nassian) as well as the optimal timing (based on peak transmission periods) to maximize the potential impact of the intervention.

1439

LABORATORY DEMONSTRATION OF TRANSOVARIAL TRANSMISSION OF RIFT VALLEY FEVER VIRUS IN *CULEX TARSALIS* MOSQUITOES

Nicholas A. Bergren, Erin M. Borland, Daniel A. Hartman, **Rebekah C. Kading**

Colorado State University, Fort Collins, CO, United States

Rift Valley fever virus is an emerging mosquito-transmitted virus with a demonstrated ability to invade previously naïve geographic areas. Current dogma, based on limited field evidence, is supportive of RVFV being maintained by transovarial transmission (TOT) from parent mosquito to offspring. Understanding the vertical transmission dynamics of RVFV is crucial for determining the risk of RVFV establishment in mosquito populations of the United States and anticipating vector control and surveillance needs. However, demonstration of vertical transmission of RVFV in the laboratory in any mosquito vector species is lacking. To address this important knowledge gap, we orally challenged *Culex tarsalis* mosquitoes (KNWR strain) with an epidemic strain of RVFV from Kenya, and tracked infection rates in progeny over three consecutive gonotrophic cycles. Progeny from all three gonotrophic cycles were reared to adults, with representatives from each developmental stage assayed for the presence of infectious virus by plaque assay. The infection rate of the parental generation was 72.0%. Infectious virus was recovered from ovarian tissues of parental mosquitoes after the first infectious blood meal. Infection via TOT was confirmed in progeny after the first ovarian cycle, with infection rates of F1 adult mosquitoes from different gonotrophic cycles ranging from 2.0-10.0%. Infectious virus was also recovered from the ovaries and salivary glands of adult F1 progeny. Virus titers were significantly lower in progeny than in parental mosquitoes, suggesting there may be some host mechanism suppressing replication. These data confirm that RVFV can be passed transovarially in *Cx. tarsalis* mosquitoes. The relative contribution of TOT for promoting the establishment of RVFV in native mosquito populations in the United States is unknown.

1440

ASSESSING ULTRA-FINE-SCALE FACTORS TO IMPROVE HUMAN WEST NILE VIRUS DISEASE MODELS IN THE CHICAGO AREA

Johnny Uelmen[1], Patrick Irwin[2], William Brown[1], Surendra Karki[1], Marilyn O'Hara Ruiz[1], Bo Li[1], Rebecca Smith[1]

[1]*University of Illinois, Urbana, IL, United States,* [2]*Northwest Mosquito Abatement District, Wheeling, IL, United States*

Since 1999, West Nile virus (WNV) has moved rapidly across the United States, resulting in tens of thousands of human cases. Both the number of human cases and the level of mosquito infection (MIR) vary across time and space and are related to numerous abiotic and biotic forces, ranging from differences in microclimates to socio-demographic factors. Because the interactions among these multiple factors affect the locally variable risk of WNV illness, it has been especially difficult to model human disease risk across varying spatial and temporal scales. Cook and DuPage Counties, comprising the city of Chicago and surrounding suburbs, are among the areas hardest hit by WNV in the United States. Despite active mosquito control efforts, there is consistent annual WNV presence, resulting in more than 285 confirmed WNV human cases and 20 deaths in the past 5 years in Cook County alone. A previous WNV model for the greater Chicago area identified the fifty-five most high and low risk study areas in the Northwest Mosquito Abatement District (NWMAD), an enclave ¼ the size of the previous study area. In these locations, human WNV risk was stratified by strength of predictive success, as indicated by differences in studentized residuals. Within these areas, an additional two-years of field collections and data processing was added to a 10-year WNV dataset and assessed by an ultra-fine-scale multivariate logistic regression. Multivariate statistical approaches revealed that this ultra-fine-scale model resulted in fewer explanatory variables while improving upon the fit of the existing model. Beyond mosquito infection rates and climatic factors, efforts

to acquire additional covariates only slightly improve model predictive performance. These results suggest human WNV illness in the Chicago area may be associated with fewer, but increasingly critical, key variables at finer scales. Given limited resources, this study suggests a large variation in the significance to model performance and provides guidance in covariate selection for optimal WNV human illness modeling.

1441

EFFECTS OF HETEROGENEOUS MICROCLIMATE TEMPERATURES ON THE RNA INTERFERENCE PATHWAY OF *AEDES AEGYPTI*

Tyler Pohlenz, Byul Hur, Madhav Erraguntla, Mark Lawley, Mustapha Debboun, Jeremy Vela, Chris Fredregill, Martin Reyna, Zach Adelman, Kevin Myles

Texas A&M University, College Station, TX, United States

Our ability to develop predictive models capable of accurately forecasting epidemics of mosquito-borne viral diseases is limited by, among other things, a relatively poor understanding of the abiotic factors influencing the dynamics of virus transmission. In particular, there is a paucity of information regarding how the spatial temperature ranges occurring in the heterogeneous microclimate environments where ectothermic mosquitoes breed and rest affect vector competence. To begin exploring how the exposure of vector species to heterogeneous microclimates might influence disease transmission, we conducted a series of experiments to determine if *Aedes aegypti* held under different constant temperatures during various developmental stages exhibited evidence of an impaired RNA interference (RNAi) pathway, the primary antiviral defense of the insect. Interestingly, we demonstrated inhibition of RNAi in *Ae. aegypti* adults reared at temperatures similar to those commonly recorded in microhabitats near mosquito trap sites located throughout urban areas of Harris County, Texas. Further, exposure of only the aquatic pupal stage to lower temperatures significantly impaired the RNAi pathway in adults. As pupal development typically takes only a few days, and pupae are confined to a specific microclimate, typically a small container of water, these results may have significant implications for understanding how the thermal environment of the vector influences disease transmission. Overall, these results suggest that exposure of *Ae. aegypti* to low diurnal temperatures over relatively short durations, particularly during the relatively immobile aquatic stages of development, may compromise the antiviral immunity of this important disease vector, increasing the potential for virus transmission.

1442

FOLLOW UP OF CHILDREN ANTIGEN-POSITIVE FOR LYMPHATIC FILARIASIS IDENTIFIED DURING A TRANSMISSION ASSESSMENT SURVEY IN HAITI

Marisa A. Hast[1], Alain Javel[2], Eurica Denis[2], Kira A. Barbre[3], Jonas Rigodon[4], Keri Robinson[1], Katherine Gass[3], Christine Dubray[1]

[1]*CDC, Atlanta, GA, United States,* [2]*IMA World Health, Port-au-Prince, Haiti,* [3]*Task Force for Global Health, Atlanta, GA, United States,* [4]*CDC, Port-au-Prince, Haiti*

The transmission assessment survey (TAS) is a standardized WHO decision-making tool to determine when the prevalence of lymphatic filariasis (LF) has reached low enough levels that mass drug administration (MDA) can be stopped. In Haiti, the threshold to stop MDA is when LF antigenemia falls below 2% among children aged 6-7 years old. A challenge facing LF programs is how to respond to antigen-positive children identified during TAS that passed this threshold. Haiti's Nippes Department passed TAS 1 and stopped MDA in 2015. Nippes passed TAS 2 in 2017; however, the number of antigen-positive children increased from 2 to 8, and 4 of these were in a single administrative commune. In 2019, a household survey was conducted in Nippes using two sampling strategies to help provide guidance for post-TAS surveillance. First, the 50 closest households to each LF antigen-positive child (index case) were selected purposively for inclusion in the survey. Twenty households were then randomly selected from each index case's census enumeration area (EA). All consenting household members ≥ 2 years old were administered a survey and asked to provide 75µl of blood by finger stick for rapid antigen testing. Overall, 29 of 1,936 (1.5%) survey participants from 794 households were antigen-positive. Higher positivity rates were identified in participants >10 years old, however two sampled children <10 years old were antigen-positive, suggesting recent transmission. Positivity rates were similar between males and females. Households selected using random sampling identified a smaller number of cases but a higher positivity rate (n=9, 2.5%) compared to households selected using purposive sampling (n=17, 1.4%). Antigen positivity was higher in the commune with 4 index cases (2.4%) than in the other participating communes (0-1.8%) (P=0.002). Overall, a substantial number of LF cases were identified through both sampling methods indicating potential ongoing transmission in this area despite passing TAS. These results demonstrate the need for systematic post-MDA surveillance and policy guidelines for responding to antigen-positive children identified in TAS.

1443

INCREASED BENEFIT OF SEMI-ANNUAL TREATMENT WITH ALBENDAZOLE ALONE TO CLEAR INDIVIDUAL INFECTION WITH *WUCHERERIA BANCROFTI*, WHEN COMPARED WITH ANNUAL TREATMENT: LONGITUDINAL ANALYSIS FROM TWO COHORT DATA FROM CENTRAL AFRICA

Jérémy T. Campillo[1], Naomi P. Awaca-Uvon[2], Jean-Paul Tambwe[2], Godé Kuyangisa Simuna[2], Gary J. Weil[3], Michel Boussinesq[1], Cédric B. Chesnais[1], Sébastien D. Pion[1]

[1]*Institut de Recherche pour le Développement, Montpellier, France,* [2]*Ministry of health, Kinshasa, Democratic Republic of the Congo,* [3]*Washington University School of Medicine, St. Louis, MO, United States*

Two community trials conducted from 2012 to 2019 in the Republic of Congo and Democratic Republic of Congo demonstrated the efficacy of semi-annual mass treatment with albendazole (ALB) alone on lymphatic filariasis (LF). However, a high inter-individual heterogeneity in the clearance of infection was observed. We used the trials data to assess the effect of individual adherence to ALB treatment on the rate of disappearance of circulating filariasis antigens (CFA) and of *Wuchereria bancrofti* microfilariae (mf). Participants were invited to be tested for LF with a rapid test for CFA at baseline and prior to each annual parasitological assessment. Results were recorded semi-quantitatively (scores at 0, 1, 2 or 3 according to the test lines intensities). Each CFA-positive individual was resampled by night to assess microfilaremia in calibrated blood smears. Participants were offered a single dose of ALB (400 mg) every 6 months. All CFA-positive subjects for whom at least one follow-up measure was available were included in the analyses. Parametric survival models were used to assess the influence of compliance to ALB treatment on CFA and mf clearance in individuals. From the 2658 subjects included in the trials, 394 (1369 person-years; PY) and 129 (400 PY) were eligible for the CFA and the mf analyses, respectively. CFA disappeared after an average of 3.9 years in those who had taken 2 doses of ALB each year; the time increased to 4.3 (P<0.001) and 5.3 (P<0.001) years in subjects who had taken 1 and 0 dose/year, respectively (adjusted on age, sex and initial CFA). Times to CFA clearance were longer in individuals with higher initial CFA test scores. Achievement of mf clearance showed a similar profile: individuals who had taken 2 doses of ALB each year became amicrofilaremic after 3.1 years, whereas those who had taken 1 or 0 dose/year needed 3.6 (P=0.008) and 5.9 years (P<0.001), respectively. Our results quantify the dose-response effect of ALB on CFA and mf clearance. Social mobilization emphasizing the importance of compliance to accelerate LF elimination should be started early and reinforced periodically with positive feedback to combat program fatigue.

1444

ONCHOCERCIASIS TRANSMISSION LIKELY INTERRUPTED IN MUCH OF THE REMAINING ACTIVE ENDEMIC AREA IN THE AMERICAS

Lindsay Rakers[1], Mauricio Sauerbrey[2], Carlos Botto[3], Andreia de Padua Careli Dantas[4], Oscar Noya-Alarcón[3], João Luiz Pereira De Araujo[4]

[1]*The Carter Center, Atlanta, GA, United States,* [2]*Onchocerciasis Elimination Program for the Americas, Guatemala City, Guatemala,* [3]*CAICET/UCV/CMT, Puerto Ayacucho, Bolivarian Republic of Venezuela,* [4]*DEVIT/SVS/MS, Brasília, Brazil*

The Onchocerciasis Elimination Program for the Americas (OEPA) regional initiative has been working to eliminate river blindness from Central and South America since 1993. Four of the six countries in the region that were originally endemic for onchocerciasis (Colombia, Ecuador, Mexico and Guatemala) have received verification of disease elimination by WHO (in 2013, 2014, 2015 and 2016, respectively). The remaining two countries where transmission continues are Brazil and Venezuela, in the shared indigenous Yanomami population that spans the countries' border in the Amazon rainforest. Just 34,000 people remained at risk in this endemic focus in 2019. Despite the intense challenges both countries face in reaching and providing treatment to this remote and migratory population, many communities in this focus have received years of regular treatment up to four times per year with ivermectin (Mectizan®, donated by Merck). Based on experiences elsewhere in the region, 20 or more rounds of consecutive, effective treatment coverage (85% or higher) suggest that transmission interruption is likely in much of this endemic focus, with 61% of the communities (384 of 629) having achieved 20 or more effective treatment rounds as of 2019 reports. On the Brazil side, this amounts to 163 communities that include 10,461 of the 16,985 persons at risk. On the Venezuela side, it is 221 communities that include 11,060 of the 16,761 persons at risk. Vector *Simulium* species PCR results for *Onchocerca volvulus* (OV) infection from both countries support the analysis of effective treatment rounds. OV-150 PCR analysis of 84,247 flies collected from five subareas in Venezuela between 2006 and 2019 and 76,353 flies collected from 3 subareas in Brazil between 2017 and 2018 fell at or below the WHO 95% CI of 0.1% threshold deemed necessary to break transmission.

1445

DISPERSAL OF *SIMULIUM* VECTORS AND ITS EFFECT ON ONCHOCERCIASIS ELIMINATION DEMYSTIFIED. LESSONS FROM THE ELIMINATION PROGRAMS

Moses Katabarwa[1], Peace Habomugisha[2], Zerihun Tadesse[3], David Oguttu[4], Aderajew Mohammed[3], Edson Byamukama[2], Abebual Yilak[3], Tewodros Seid[3], Kadu Meribo[3], Lauri Bernard[1], Emily Griswold[1], Frank O. Richards[1]

[1]*The Carter Center, Atlanta, GA, United States,* [2]*The Carter Center, Kampala, Uganda,* [3]*The Carter Center, Addis Ababa, Ethiopia,* [4]*Ministry of Health, Vector Control Division, Kampala, Uganda*

The age-old belief that *Simulium* vectors' flight range can extend as far as 160 km (100 miles) meant that onchocerciasis transmission zones are very large and difficult to eliminate. *Simulium* flies can disperse from other transmission zones and reseed those areas where success had been attained. Thus, this belief promotes skepticism among donors and decision-makers, that onchocerciasis elimination is not a realistic goal. We applied epidemiological and entomological information from onchocerciasis foci in Ethiopia and Uganda, including the Wude Gemuz hotspot as a proxy for determining *Simulium* flight ranges. The buffer zone between the Galabat-Metema focus (*S. damnosum*) and Metekel focus (*S. damnosum*) to the south and Wude Gemuz Hotspot within Metema Subfocus were examined for the vector flight range. In Uganda, we examined the buffer zones between the Bwindi focus (*S.neavei*) and nearby *S.damnosum* breeding sites; Nyamugasani and Lhubiriha foci (*S.kilbarnum*); Wambabya-Rwamarongo focus (*S.neavei*) and Budongo focus (*S.neavei*); and Madi Mid North focus and to the south, Kiryandongo area (*S.damnosum)*. Where two foci were neighboring, we assessed the buffer zone between them for any evidence of *Simulium* vectors crossing to infect the next focus, before interventions were halted. The results showed that *Simulium* vectors flight ranges from their breeding sites varied from three to about ten kilometers. In Galabat-Metema, where vector control was not done, the buffer zone with the Metekel Zone was at least 20 km wide. There was no evidence of possible invasion of flies from Metekel focus through the buffer zone to the southern limits of Galabat-Metema focus. Based on these results, Galabat-Metema passed the WHO criteria for transmission interruption and interventions were halted and the focus moved to a three-year Post Treatment Surveillance (PTS) period. In some cases, *S.damnosum* behaved like *S.neavei*. We concluded that *Simulium* vectors including *S.damnosum* that were investigated had different dispersal distances within the 20 km range.

1446

A GEOSPATIAL ANALYSIS OF THE IMPACT OF INTERVENTION ON ONCHOCERCIASIS ENDEMICITY IN CÔTE D'IVOIRE

Obiora Eneanya[1], Benjamin G. Koudou[2], Yeo Souleymane[3], Marie-Madeleine Kouakou[4], Peter U. Fischer[1], Gary J. Weil[1]

[1]*Washington University, St. Louis, MO, United States,* [2]*Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, Abidjan, Côte D'Ivoire,* [3]*Ministry of Public Health and Hygiene,, Abidjan, Côte D'Ivoire,* [4]*Ministry of Public Health and Hygiene, Abidjan, Côte D'Ivoire*

Côte d'Ivoire has had 45 years of intervention to control onchocerciasis by vector control (from 1975 to 1991) and community directed treatment with ivermectin (CDTi, since 1992). The purpose of this study was to model onchocerciasis endemicity before and after CDTi, to review the impact of intervention and evaluate the feasibility of elimination. Our analysis was based on microfilaria (mf) prevalence by skin snip and community mf load (CMFL) data before (1975-1991) and during (1992-2016) CDTi. Socio-demographic and environmental factors were incorporated into a predictive, machine learning algorithm to create continuous maps of onchocerciasis endemicity. Mean Mf prevalence in endemic regions decreased between baseline to 2016 from 51.8% to 3.9%, although limited meso-endemic foci remain in the southern region. Predicted mean CMFL in endemic regions decreased from 10.1mf/snip to 0.1mf/snip, although limited foci in the southern region are predicted to still have >5mf/snip. For assessing model performance, the root mean squared error and R-squared values were 1.32 and 0.58 respectively. The mean Pearson's correlation between observed and predicted prevalence at validation locations was 0.638. Finally, our models show that proximity to inland streams, rivers and coastline, and altitude were the most informative variables that correlated with endemicity. This study documents the significant impact of interventions on the prevalence and intensity of onchocerciasis in Côte d'Ivoire. Maps produced have delineated remaining foci of infection. With sustained treatment coverage, adequate surveillance, and close monitoring of infection status in previous hyper-endemic areas, we believe that onchocerciasis transmission can be interrupted in the future. Although this study focused on Côte d'Ivoire, this approach may be useful for identifying persistent endemic areas and targeting interventions to eliminate onchocerciasis across Africa.

1447

A COMPARISON OF ONCHOCERCIASIS SEROLOGICAL TOOLS AND POTENTIAL APPLICATION FOR PROGRAM USE

Eric Scott Elder[1], Holly Chastain[1], Henry M. Kanyi[2], Sammy M. Njenga[2], Joseph Kamgno[3], Laston Sitima[4], Benjamin A. Marfo[5], Guilherme M. Ogawa[1], Vitaliano A. Cama[1], W. Evan Secor[1], Kimberly Y. Won[1]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Kenya Medical Research Institute (KEMRI), Nairobi, Kenya,* [3]*Centre for*

Research on Filariasis and other Tropical Diseases (CRFilMT), Yaounde, Cameroon, [4]Community Health Sciences Unit (CHSU), Lilongwe, Malawi, [5]Ghana Health Service, Accra, Ghana

Standardized, high quality diagnostic tools are critical for appropriate intervention decisions in onchocerciasis elimination programs. One testing approach under evaluation involves detection of antibodies to the Ov16 antigen. To this end, we compared two ELISA platforms, the Standard Diagnostics (SD) Onchocerciasis IgG_4 ELISA and an alkaline phosphatase (AP) ELISA developed at CDC, and one lateral flow assay (SD Oncho/LF IgG_4 Biplex) using Dried Blood Spots (DBS) in laboratories in the United States, Cameroon (CRFilMT) and Kenya (KEMRI). Positive control sera obtained from persons from *Onchocerca volvulus* endemic areas who were skin snip positive for microfilariae were used to construct reference curves and tested on each ELISA platform multiple times. The SD ELISA yielded more consistent results with lower average coefficient of variation values compared with the AP ELISA, which also had a higher number of plates that were excluded because they failed quality control criteria. DBS collected in Malawi (n = 1872) and tested at KEMRI demonstrated a 14.3% positive rate by SD ELISA but only 0.4% were positive using the AP ELISA. By contrast, DBS collected in Ghana (n = 2753) and tested at CRFilMT showed a higher positivity rate in the AP ELISA (20.2%) compared to the SD ELISA (8.4%). In addition, a positive (n = 80) control DBS panel was created artificially by spiking separated red blood cells with sera and a negative (n = 99) control DBS panel was assembled with field collected samples from 2 non-onchocerciasis endemic countries. The positive panel run at CDC gave a 95.0% positivity rate on both the SD Oncho/LF Biplex and SD ELISA and a 90.0% positive rate on the AP ELISA. On the SD ELISA, 10.0% of the negative panel samples were positive while 0.0% of the samples were positive on the SD Oncho/LF Biplex. Variable performance of both ELISAs make it challenging to recommend either of these platforms for programs. More consistent results were observed with eluted DBS on the SD Oncho/LF Biplex, and may be suitable for elimination mapping. However, further evaluation and testing will be necessary before antibody detection by these ELISAs is feasible for program use.

1449

COST-BENEFIT ANALYSIS OF WOLBACHIA TO CONTROL DENGUE IN SUVA, FIJI AND PORT VILA, VANUATU

Donald S. Shepard[1], Dhwani Hariharan[1], Anaseini Ratu[2], Katherine L. Anders[3]

[1]*Brandeis University, Waltham, MA, United States,* [2]*World Mosquito Program, Suva, Fiji,* [3]*Monash University, Melbourne, Australia*

Releasing mosquitos containing *Wolbachia* bacteria is proving to be an effective control mechanism against dengue in multiple sites in Asia and South America. Modeling studies from Indonesia indicated that the technology would be highly cost effective in moderate-sized or large cities with endemic dengue, such as Yogyakarta or Jakarta. However, the economic viability of this approach in smaller cities or those less severely affected by dengue is not known. We conducted benefit-cost analysis and cost-effectiveness analyses of *Wolbachia* deployment in the largest cities of two Pacific island nations, Fiji and Vanuatu (2018 per capita Gross National Income US$4,860 and $3,130, respectively). These cities, Suva and Port Vila, had 82,000 and 44,000 inhabitants, respectively. We obtained costs of *Wolbachia* deployment from the World Mosquito Program's 2016-18 experience, baseline numbers of symptomatic dengue cases by setting and outcome from surveillance and global epidemiological data, direct and indirect cost per dengue case by setting from costing studies and experts, and effectiveness from global quasi-experimental studies. We adjusted for incomplete testing and calculated aggregate costs. On average, these cities experienced 2,500 and 605 dengue cases (437 and 11 hospitalized, 723 and 150 ambulatory, 1,341 and 445 not-medically attended, including 0.04 and 0.01 fatalities), with medical costs of $177,859 and $8,303 and aggregate economic costs of $710,442 and $71,141 annually, respectively. Establishing *Wolbachia* cost $2,724,438 and $2,404,545, respectively, and is estimated to avert 75% of the cases, deaths, and disease costs. Based on *Wolbachia's* expected 10-year lifetime, the benefit-cost and cost-effectiveness ratios were favorable in Suva (1.61:1 and $4,980/DALY averted) but not in Port Vila (0.19:1 and $20,193/DALY averted). Even if *Wolbachia* in Port Vila remained effective permanently, it would still not be cost-beneficial nor cost-effective. In middle-income countries, *Wolbachia* use appears economically viable in moderately sized cities with a high dengue burden, but not in small cities or those with lower burden.

1450

TEMPORAL PATTERNS IN SYNCHRONY IN THE DYNAMICS OF DENGUE: THE ROLE OF TEMPERATURE AND IMMUNITY

Bernardo Garcia-Carreras[1], Angkana T. Huang[1], Bingyi Yang[1], Henrik Salje[2], Mary K. Grabowski[3], Sopon Iamsirithaworn[4], Justin Lessler[3], Derek A. Cummings[1]

[1]*University of Florida, Gainesville, FL, United States,* [2]*University of Cambridge, Cambridge, United Kingdom,* [3]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [4]*Thailand Ministry of Public Health, Nonthaburi, Thailand*

The spatial distribution of dengue and its mosquito vectors (spp. *Aedes*) is the widest it has ever been, and projections suggest that ongoing climate change allows the expansion to continue. However, the largest overall impacts of climate change on dengue might be in regions where the pathogen is already endemic, areas which even in long-term projections, comprise a majority of the world's population at risk of dengue. The environment, and particularly temperature, by altering mosquito life history traits, influences dengue transmission rates, and thus has the potential to affect the dynamics of dengue cases. In this study, we characterise spatio-temporal patterns of dengue in Thailand, where dengue has caused almost 1.5 million cases over the last thirty years, and examine the roles played by temperature and dynamics of immunity in giving rise to those patterns. Using multiple approaches, we uncover an interesting spatial phenomenon in dengue: while the dynamics can differ and be quite heterogeneous across the country, at certain points in time their dynamics synchronise and become more homogeneous, i.e., Thailand has been experiencing periodic synchronizations in dengue cases. When analysing temperature time series across Thailand, we find a similar pattern in spatial synchrony, implying the likely importance of temperature in generating the observed patterns. Aside from temperature, immunity is central in shaping dengue dynamics; temporary cross-protection between serotypes alone can give rise to a qualitatively wide range of dengue dynamics. Thus, any effects of temperature on dynamics must necessarily be modulated by immunity. To gain a more mechanistic understanding of how temperature and dynamics of immunity interact to give produce the observed patterns in synchrony, we adapted a mechanistic, temperature-dependent, four-serotype dengue model with temporary cross-protection. We show, using simple experiments, how periodic synchronisation of a system can be achieved, and find that the relationship between temperature and dengue dynamics is made more complex with increasing levels of cross-protection.

1451

TEMPORALLY INTEGRATED SINGLE CELL RNA SEQUENCING ANALYSIS OF CONTROLLED AND NATURAL DENV-1 INFECTIONS

Adam Waickman[1], Heather Friberg[1], Gregory D. Gromowski[1], Wiriya Rutvisuttinunt[1], Tao Li[1], Hayden Siegfried[1], Kaitlin Victor[1], Caitlin Kuklis[1], Michael K. McCracken[1], Stefan Fernandez[2], Anon Srikiatkhachorn[3], Damon Ellison[1], Richard G. Jarman[1], Stephen J. Thomas[4], Alan L. Rothman[3], Timothy Endy[4], Jeffrey R. Currier[1]

[1]*Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [2]*Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand,* [3]*University of Rhode Island, Providence, RI, United States,* [4]*State University of New York, Upstate Medical University, Syracuse, NY, United States*

Controlled dengue human challenge studies present a unique opportunity to address many longstanding questions in the field of flavivirus biology. These fundamental questions include defining the early immunological

signatures of infection, the host/environmental factors that impact disease severity, and the role of preexisting immunity on the development of symptomatic viral infection. However, while several controlled dengue human challenge studies have been performed and appear to clinically recapitulate may features of mild natural DENV infection, limited data are available on how the immunological and transcriptional response elicited by these attenuated challenge viruses compares to the profile associated with a natural, wild-type DENV infection. To bridge this knowledge gap, we performed scRNAseq analysis on longitudinally collected PBMC samples obtained from 3 individuals (8 time-points per subject) enrolled in the SUNY/WRAIR DENV-1 controlled human challenge study. In addition, 3 time-points (two acute infection time-points, one control time-point) from two individuals experiencing a natural DENV-1 infection were analyzed and integrated with the challenge model dataset. This dataset contains a total of 171,208 cells and 22 distinct populations corresponding to all major leukocyte subsets. While all identified cell populations demonstrated significant and consistent perturbations in their transcriptional profile in response to either natural or experimental DENV infection, conventional monocytes responded most robustly to infection across all subjects and study groups from an unbiased transcriptional perspective. Using these data, core sets of genes that were consistently induced by either natural or experimental DENV were identified, and the overlap between the two arms of the study were assessed. The gene set associated with experimental DENV infection was found to reflect a subset of genes within the larger gene set associated with natural DENV infection. These data provide insight into the molecular level response to DENV infection, and how viral pathogenesis correlates with immune activation.

1452

PROFILING OF THE EPITOPE DIVERSITY AND EVOLUTION OF DENGUE BINDING ANTIBODIES BY PEPTIDE MICROARRAY

Francesca Falconi-Agapito[1], Karen Kerkhof[1], Xiomara Merino[2], Marjan Van Esbroeck[3], Michael Talledo[2], Kevin K. Ariën[1]

[1]*Virology Unit, Institute of Tropical Medicine, Antwerp, Belgium,* [2]*Virology Unit, Instituto de Medicina Tropical Alexander von Humboldt, Lima, Peru,* [3]*Department of Clinical Sciences, National Reference Center for Arboviruses, Institute of Tropical Medicine, Antwerp, Belgium*

Infections with arthropod-borne viruses are increasing globally. The similar symptomatology of arboviral diseases and the co-circulation of different arboviruses in Africa, Asia and South America complicate diagnosis. The high genetic and antigenic similarity, especially among the flavivirus family, leads to the detection of cross-reactive antibodies (Abs) by the commercially available tests. On this regard, finer methods are needed, first to characterize the epitope diversity of the Ab response; and secondly, to identify antigenic regions that can be recognized by type-specific Abs. In this study, we designed a high-density peptide microarray for the measurement of the Ab diversity against 9072 linear epitopes covering the entire proteome of dengue, zika, yellow fever and chikungunya viruses. Using this microarray, we quantified the IgG binding response in dengue infected individuals from Peru and from overseas travelers returning to Belgium, here considered as secondary and primary infections, respectively. Four serum samples were collected over the period of six months in patients from Peru and two samples within 14 days after onset of symptoms in the travelers. We found common and differing patterns of epitope-specificity between primary and secondary infections. The magnitude of the Ab response in both groups was higher for peptides located in discrete regions in the capsid, envelope, NS1, NS4A, NS3 and NS5. Some of these peptides were consistently targeted over the course of the infection, while for other peptides this reactivity changed over time. We found that early in the infection, the number of unique epitopes targeted ("Ab breadth"), and, the number of epitope variants recognized ("Ab depth") was high. This Ab response stabilized or declined over the course of the infection. The Ab breadth was higher in primary (n=35) compared to secondary (n=25) infections, this was specially the case for the envelope, NS3 and NS5 proteins. The mapped epitope regions will be selected as biomarkers to design better serologic tools for arboviral diagnosis.

1453

OBESITY AND THE INCREASED RISK OF SEVERE DENGUE: POSSIBLE PATHOPHYSIOLOGICAL MECHANISMS

Peter B. Gallagher, Nguyet M. Ngyuen, Huynh T. Duyen, Nguyen L. Vuong, Tran T. Vi, Nguyen T. Kieu, Huynh L. Huy, Phung K. Lam, Dong T. Tam, Sophie Yacoub

Oxford University Clinical Research Unit, Ho Chi Minh City, Viet Nam

Dengue is a neglected tropical disease that causes an estimated 100 million clinically apparent infections, with as many as 4 billion people being at risk of infection. While most dengue cases present as a self-resolving febrile illness, a small percentage of cases progress to severe disease that requires intensive care and can be fatal. While some risk factors have been identified for disease progression, recently obesity has been identified as a risk factor for severe disease, however, there is a paucity of mechanistic data to explain this increased risk. With increasing rates of obesity in dengue endemic areas, it is important to understand the pathophysiological mechanisms underlying this increased risk as well as further our understanding of dengue pathology. We are conducting a matched-cohort study of 150 dengue patients at the Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam. 75 patients will be enrolled into each arm: obese/overweight (BMI >30/>25 respectively) and a control arm (BMI <22). A range of biomarkers known to be involved in the pathology of dengue and/or obesity are being measured at serial intervals during the illness as well as detailed clinical data recorded. Biomarkers include- inflammatory, adipokines, vascular activation and damage. 114 patients have been enrolled so far (23 Obese, 34 Overweight, and 57 Controls). Interim analysis shows that there is a significant increase in the concentrations of markers of inflammation in the obese/overweight group (CRP and Ferritin, p= 0.046/0.001 respectively) and that markers of endothelial activation (VCAM1, Ang-2, etc) are associated with severe disease. However, no link was shown between the concentration of adipokines and severe disease suggesting specific inflammatory pathways are activated in these cases. Further studies are being performed including AMPK activation and immunological profiling. This study will provide novel information on the mechanisms underlying the increased risk of severe dengue found in obese patients. This will allow us to identify new potential prognostic markers as well as novel pathways for targeted treatment of this high-risk group.

1454

STRUCTURAL BASIS FOR ANTIBODY-MEDIATED INHIBITION OF FLAVIVIRUS NS1-TRIGGERED ENDOTHELIAL DYSFUNCTION

Scott B. Biering[1], David L. Akey[2], Marcus P. Wong[1], William Clay Brown[2], Nicholas TN Lo[1], Henry Puerta-Guardo[1], Chunling Wang[1], Jamie R. Konwerski[2], Francielle Tramontini Gomes de Sousa[1], Diego A. Espinosa[1], Dustin R. Glasner[1], Jeffrey Li[1], Sophie F. Blanc[1], Stephen J. Elledge[3], Michael J. Mina[4], P. Robert Beatty[1], Janet L. Smith[2], Eva Harris[1]

[1]*Division of Infectious Diseases & Vaccinology, School of Public Health, University of California, Berkeley, Berkeley, CA, United States,* [2]*Life Sciences Institute, University of Michigan, Ann Arbor, MI, United States,* [3]*Harvard Medical School, Boston, MA, United States,* [4]*Harvard School of Public Health, Boston, MA, United States*

Medically important flaviviruses such as dengue virus (DENV) are responsible for a major global disease burden and cause diverse disease pathologies broadly manifesting in endothelial dysfunction. A contributing factor to these pathologies is secreted flavivirus non-structural protein 1 (NS1), which can directly trigger endothelial hyperpermeability and vascular leak. Despite demonstrations of anti-NS1 antibody-mediated protection against lethal flavivirus challenge, the structural and mechanistic basis of this protection remain obscure. Here, we solved two crystal structures of DENV1 and DENV2 NS1 complexed with the Fab fragment of a flavivirus cross-reactive NS1-specific monoclonal antibody, 2B7, pointing to a protective mechanism by which two domains of NS1 are antagonized

simultaneously. Specifically, 2B7 binds directly to the C-terminal β-ladder of NS1 which, in addition to obscuring this domain, also results in steric hinderance of the NS1 wing domain. Utilizing NS1 mutants located in the 2B7 epitope within the β-ladder as well as point mutants in the wing domain, we further demonstrate that the wing domain of NS1 mediates binding to endothelial cells while the β-ladder mediates steps downstream of cell binding, both of which are necessary for triggering endothelial dysfunction. The NS1:2B7 combining site contains two groups of amino acids: a flavivirus-conserved internal core and a divergent peripheral region, which reflects the binding capacity of 2B7 to diverse flavivirus NS1 proteins. Further, our data indicate that 2B7 inhibits NS1-mediated endothelial hyperpermeability from other flaviviruses, including Zika and West Nile viruses. Thus, we provide a structural and mechanistic explanation for 2B7 protection against NS1-induced pathology and demonstrate the potential to treat multiple flavivirus infections with one monoclonal antibody.

1455

CYCLODEXTRINS INHIBIT DENGUE VIRUS NONSTRUCTURAL PROTEIN 1-MEDIATED ENDOTHELIAL DYSFUNCTION

Francielle Tramontini Gomes de Sousa[1], Trishna Patel[1], Peter Laing[2], Tamás Sohajda[3], Robert Beatty[1], Eva Harris[1]

[1]*Division of Infectious Diseases & Vaccinology, School of Public Health, University of California, Berkeley, Berkeley, CA, United States,* [2]*Excivion Ltd, Cambridge, United Kingdom,* [3]*CycloLab, Cyclodextrin R&D Laboratory, Budapest, Hungary*

Severe dengue virus (DENV) infections are characterized by increased vascular permeability and hemorrhagic manifestations. Despite its substantial morbidity and mortality, no therapeutic agents exist for treatment of dengue, and the currently available vaccine does not confer full protection. Thus, development of therapeutic and/or preventive drugs is urgently needed. Nonstructural protein 1 (NS1) plays important roles in host immune evasion and viral pathogenesis by directly triggering endothelial barrier dysfunction and inducing inflammatory responses, contributing to vascular leak *in vivo*. Cyclodextrins (CDs) are a family of cyclic oligosaccharides largely used as drug excipients but also as active pharmaceutical ingredients. Further, CDs have been shown to possess broad-spectrum antiviral activity against human immunodeficiency virus, herpes simplex virus, influenza virus, Zika virus, and DENV, with mechanisms of action including inhibition of viral entry and replication, as well as cholesterol-sequestering and virucidal activity. Here, we evaluated the *in vitro* efficacy against DENV NS1-mediated pathogenesis of a series of 20 different CDs that display variable cavity sizes as well as degree and type of substitution. In an *in vitro* model of endothelial permeability, CDs, at concentrations that had zero anticoagulant effect, were added to human pulmonary microvascular endothelial cells (HPMECs) in the presence of DENV2 NS1. Endothelial dysfunction was quantified by measuring Trans-Endothelial Electrical Resistance (TEER). CDs labelled as 2, 5, and 17 (at 100, 100, and 10 ug/mL, respectively) significantly reduced TEER values when compared to NS1-only treatment. No cytotoxicity was observed for any CD tested up to 1500 ug/mL. Sulfation substitution contributed to the anti-leak activity. We are currently assessing the inhibition of NS1 binding to HPMECs by CDs, their *in vitro* anti-DENV activity, and their *in vivo* efficacy in murine models of vascular leak. These findings hold promise for the use of sulfated glycans for dengue treatment, alone or in combination with compounds having different mechanisms of action.

1456

HTRACK: A NEW TOOL TO FACILITATE PUBLIC HEALTH FIELD VISITS AND ELECTRONIC DATA CAPTURE

Dania Rodriguez, Laura Adams, Ryff Kyle, Gabriela Paz-Bailey

Centers for Disease Control and Prevention, San Juan, PR, United States

Community based surveys often require navigating field sites to identify selected households, monitoring contact attempts, and capturing visit outcomes. However, limited software tools are available to provide this functionality offline and comply with health and human services data security requirements. To address this, we created an R-Shiny Household-Tracking application (app), HTrack, for use on mobile devices in the field. HTrack was implemented in the Communities Organized to Prevent Arboviruses (COPA) project, a community-based cohort study initiated in 2018 to study arboviral incidence in Ponce, Puerto Rico. This study includes selecting a random sample of households in 38 communities and conducting multiple visits for participant recruitment. The app includes a live map for GPS navigation, a data entry area, a searchable data table, and a statistics area to capture cumulative progress. Data are stored locally on the device and update the map in real-time. Data transfer is performed through secure servers. Among selected households, we calculated response rate (% recruited among all visited), refusal rate (% declining to participate among all visited) and cooperation rate (% recruited among those contacted). Ineligible households were excluded. During April 2018-May 2019, HTrack was used by 30 field staff to visit a total of 23,833 households and capture information about the structure type, eligibility criteria, and field notes. Households eligible for enrollment had at least one inhabitant ≤50 years of age. HTrack was used to schedule visits when requested. We made 45,907 visits with HTrack, including 6,378 (13.9%) scheduled and 39,529 (86.1%) unscheduled visits. We found a 22% response rate, 19% refusal rate, and 52% cooperation rate. This application facilitated rapid and accurate data capture and provided for near real-time data crucial for assessing field logistics, recruitment status and project monitoring. This customizable data collection tool makes HTrack an ideal solution for field studies requiring household-level navigation and visit-tracking and is available by request from CDC Dengue Branch.

1457

EVALUATION OF A MOBILE HEALTH (MHEALTH) WEARABLE DEVICE SYSTEM FOR SEPSIS MONITORING IN GHANA

Stephanie C. Garbern[1], George Oduro[2], Christopher Oppong[2], Stephan W. Wegerich[3], Carolyn Baer[4], Charmagne G. Beckett[5], Benjamin Espinosa[6], Adam C. Levine[1], Danielle Clark[4]

[1]*Warren Alpert Medical School, Brown University, Providence, RI, United States,* [2]*Komfo Anokye Teaching Hospital, Kumasi, Ghana,* [3]*PhysIQ, Chicago, IL, United States,* [4]*Austere environments Consortium for Enhanced Sepsis Outcomes (ACESO), Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, MD, United States,* [5]*Naval Medical Research Center, Infectious Diseases Directorate, Silver Spring, MD, United States,* [6]*Naval Medical Research Center, Biological Research Directorate, Frederick, MD, United States*

Low- and middle-income countries (LMICs) bear a disproportionately high burden of sepsis, contributing an estimated 90% of global sepsis-related deaths. Critical care capabilities needed for septic patients, such as continuous vital sign monitoring, are often unavailable in LMICs. This study aimed to assess the feasibility and accuracy of using a wireless, wearable biosensor device linked to a smartphone for continuous vital sign monitoring in emergency department (ED) patients with suspected sepsis in Ghana. This was a prospective observational study of adult ED patients (≥18 years) with suspected sepsis presenting to the Komfo Anokye Teaching Hospital (KATH) in Ghana. Wearable devices were applied to patients' anterior upper left chest to continuously recorded vital signs (including heart rate and respiratory rate) for up to 72 hours and compared to intermittent manually collected vital signs every 8 hours. Pearson's correlation coefficients were calculated over the study population to determine the correlation between the vital signs obtained from the biosensor device and those manually collected. A total of 61 patients were enrolled from June 2019-February 2020 with a total of 420 pairs of vital sign measurements for comparison. Wearable device and manual vital signs were strongly correlated for heart rate ($r=0.92$, $p<0.001$) respiratory rate ($r=0.86$, $p<0.001$), and calibrated skin temperature ($r=0.81$, $P<0.001$). Mean absolute (SD) differences (bias) between wearable device and manual measurements were 2.32 (6.53) beats/min, 1.89 (2.95) breaths/min and 0.45 (0.41) degrees Celsius. Minor technical or practical feasibility

issues occurred in 5 patients (8.1%) and included biosensor detachment and temporary connectivity problems. Wearable biosensor devices can be feasibly implemented and provide accurate continuous vital sign measurements in critically ill adult patients with suspected sepsis in a resource-limited setting, or in austere environments applicable to military delayed field care. Further prospective studies evaluating the impact of biosensor devices on clinical outcomes for septic patients are needed.

1458

GLOBAL VACCINE RISK INDEX A COMPOSITE METRIC TO ASSESS THE RETURN OF MEASLES FROM 21ST CENTURY SOCIAL AND PHYSICAL DETERMINANTS

Dr Peter Hotez[1], **Tasmiah Nuzhath**[2], Brian Colwell[2], Ashish Damania[1]

[1]*Baylor College of Medicine, Houston, TX, United States,* [2]*Texas A&M School of Public Health, College Station, TX, United States*

There has been a sudden and unexpected rise in the number of global measles cases and deaths. In order to understand the basis for why measles and other vaccine-preventable diseases have returned, there is an opportunity to look at declines in vaccine coverage and the return of measles more broadly by including some of these additional determinants. We explored the physical and social determinants causing variation in vaccination coverage across countries and WHO regions to calculate the vaccination risk index and heat map, which incorporates published vaccine confidence estimates, but also looks at indices related to human development, conflicts, disasters and refugee movements, and climate change metrics. All indicators were scaled from zero to 100 using the min-max rescaling method, data were imputed using multiple imputations and the vaccine risk index was calculated by taking the geometric mean of the positive factors index and subtracting it from the geometric mean of negative factors index. Vaccine risk index values were ranked in ascending order. Our study finds that developing countries are at high risk due to low vaccination coverage, lack of accessibility to vaccination due to poor infrastructure, that along with conflict, constitute the major barriers in these countries. In European and North American countries, attitudes towards vaccination as illustrated by low vaccination confidence have limited vaccination coverage. We find that such an index reflects or approximates the geographic distribution of rising measles estimates and might serve as a useful public health tool for predicting current and future measles outbreaks.

1459

MODELING THE COVID-19 OUTBREAK IN REAL-TIME - BALANCING URGENT PUBLIC-HEALTH NEEDS WITH RESEARCH INTEGRITY

Andrea Geri Buchwald[1], David M. Bortz[2], Kathryn L. Colborn[3], Elizabeth J. Carlton[1], Jonathan M. Samet[1]

[1]*Colorado School of Public Health, Aurora, CO, United States,* [2]*University of Colorado, Boulder, CO, United States,* [3]*University of Colorado, Anschutz, Aurora, CO, United States*

The ongoing COVID-19 pandemic poses a substantial challenge to policymakers and scientists alike. Public health departments have an urgent need for data to guide and support urgent decision making including the timing and stringency of social-distancing measures to curb the severity of the pandemic. Mathematical modeling has proven to be a cornerstone of public health responses at both the global, national, and local levels in reacting to this fast-developing public health challenge. However, few governments had resources in place to immediately develop, test, and implement the use of mathematical models to guide decision making. We present on the real-time urgent development of an SEIR deterministic compartmental model to predict the COVID-19 outbreak in Colorado utilizing local reporting, demographic and hospital capacity data and responding to an immediate political and policy-based need. The model structure was progressively updated to account for our rapidly changing understanding of COVID-19 such as the age-dependent onset of symptoms. The model was used to project the peak of the epidemic, the timing of ICU capacity overload, and the impact of ICU capacity on cases and deaths for an array of social distancing and other intervention scenarios. Model fitting has been used to estimate the efficacy of different social distancing policies. We have responded to frequent urgent data requests from policymakers in Colorado and aimed to adapt the model to best respond to those requests and represent the range of potential outcomes while providing actionable information specific to Colorado. The model was fit to case and hospitalization data on a weekly basis, and accounts for local demographics and hospital bed capacity. Parameters were regularly updated from the evolving body of literature on COVID-19 transmission dynamics or derived from model fitting. We will discuss the process of working with policymakers to respond to an urgent public health need, communicating the strengths and limitations of our projections, as well as the evolution of model structure and outputs in response to rapidly evolving science and real-time surveillance data.

1463

CORRELATES OF PROTECTION FOR POWASSAN VIRUS IN MOUSE MODELS OF INFECTION

E. Taylor Stone[1], Mariah Hassert[1], Gregory D. Ebel[2], Alec J. Hirsch[3], James D. Brien[1], Amelia K. Pinto[1]

[1]*Saint Louis University, Saint Louis, MO, United States,* [2]*Colorado State University, Ft. Collins, CO, United States,* [3]*Oregon Health & Science University, Portland, OR, United States*

Powassan virus (POWV) is a tick-borne flavivirus that causes fatal meningoencephalitis in 10-15% of reported human cases. According to the CDC, a dramatic spike in incidence of POWV infection in the United States has occurred in the last three years, and is expected to continue to increase as the warming climate expands the range of *Ixodes* ticks capable of transmitting the virus to humans. While effective vaccines exist for a related virus, tick-borne encephalitis virus (TBEV), multiple studies have demonstrated that these vaccines do not provide protection against POWV. Defining the correlates of protection for POWV is a critical step in the development of efficacious POWV vaccines. To this end, we used C57BL/6 mice as a model of POWV infection to determine the protective capacity of POWV-specific T cells and antibodies against lethal POWV challenge using a less virulent lineage POWV-Spooner (Lineage II) and a more virulent lineage POWV-LB (Lineage I). Using our model, we have identified two murine CD8 T cell epitopes and two CD4 T cell epitopes, one of which appears to only be found in POWV-Spooner immune mice. We also have evidence suggesting that POWV-LB specific-antibodies can cross-bind West Nile virus (WNV), however POWV-Spooner specific-antibodies could not recognize WNV. These results suggest that there are differences in the adaptive immune response against the different POWV linages, which may be critical for future vaccine development. This data reasserts the importance of establishing correlates of protection for different POWV lineages to inform vaccine design. Finally, applying our understanding of correlates of protection for POWV, we are currently in the process of testing a POWV-based virus-like particle (POWV-VLP) vaccine candidate, which we expect will provide protection against lethal POWV challenge in our model.

1464

EMERGENCE AND SPREAD OF POWASSAN VIRUS IN THE NORTHEASTERN UNITED STATES

Chantal B. Vogels[1], Erica Normandin[2], Rebecca M. Robich[3], Alan P. Dupuis II[4], Doug E. Brackney[5], Anderson F. Brito[1], Sarah Lapidus[1], Rebekah McMinn[6], Scott C. Williams[5], Charles B. Lubelczyk[3], Joseph R. Fauver[1], Heidi Goethert[7], Jody L. Gangloff-Kaufmann[8], Pardis C. Sabeti[2], Laura D. Kramer[4], Laura B. Goodman[8], Gregory D. Ebel[6], Sam R. Telford III[7], Robert P. Smith[3], Alexander T. Ciota[4], Philip M. Armstrong[5], Anne Piantadosi[9], Nathan D. Grubaugh[1]

[1]*Yale School of Public Health, New Haven, CT, United States,* [2]*Broad Institute of MIT and Harvard, Cambridge, MA, United States,* [3]*Maine*

Medical Center Research Institute, Scarborough, ME, United States, [4]Wadsworth Center, New York State Department of Health, Slingerlands, NY, United States, [5]The Connecticut Agricultural Experiment Station, New Haven, CT, United States, [6]Colorado State University, Fort Collins, CO, United States, [7]Tufts University, North Grafton, MA, United States, [8]Cornell University, Ithaca, NY, United States, [9]Emory Vaccine Center, Atlanta, GA, United States

Powassan virus is an emerging tick-borne virus of concern for public health, but very little is known about its ecology and transmission patterns. Since its initial discovery in the 1950s, yearly cases of neuroinvasive disease in humans have increased over 20-fold. Despite the growing public health concerns, particularly in the northeastern United States, Powassan virus remains a highly underrecognized human pathogen. Consequently, very little is known about how Powassan virus is locally maintained and how it spreads. The purpose of our study was, therefore, to unravel the genetic structure of Powassan virus populations in the northeastern U.S. to gain insight in local transmission patterns. Genetic approaches are a powerful tool to gain insight in pathogen transmission patterns, but until now very limited genomic data were available for Powassan virus in the Northeast. By partnering with public health institutes in Connecticut, Maine, Massachusetts, and New York we have sequenced more than 100 additional Powassan virus genomes. We reconstructed phylogenetic trees (*i.e.* determined genetic relations) and found that Powassan virus is maintained in highly segregated transmission foci. In addition, we found that Powassan virus was likely introduced in the Northeast during multiple independent events. To understand drivers of Powassan virus emergence and spread we will compare estimates of time of virus introduction with (re-)introduction of the main host (white-tailed deer) and vector (*Ixodes scapularis* ticks) in the region. Our study provides important insights in the ecology of a rapidly emerging tick-borne pathogen of public health importance. Understanding local transmission patterns will inform the scale (*i.e.* targeting all sites within transmission foci) and effective duration of transmission suppression (*i.e.* when to expect virus reintroductions) for future control programs.

1465

A NOVEL INSECT-SPECIFIC VIRUS AS A VECTOR FOR DEVELOPING FLAVIVIRUS VACCINES EMPHASIZES NEW POSSIBILITIES FOR HIGH DEGREES OF SAFETY WITHOUT SACRIFICING IMMUNOGENICITY

Danielle LaBrie Porier[1], Sarah N. Wilson[1], Dawn I. Auguste[1], Andrew Leber[2], James D. Weger-Lucarelli[1], Scott C. Weaver[3], Albert J. Auguste[1]

[1]*Virginia Polytechnic Institute and State University, Blacksburg, VA, United States, [2]Landos Biopharma Inc., Blacksburg, VA, United States, [3]University of Texas Medical Branch, Galveston, TX, United States*

Insect-specific viruses, particularly insect-specific flaviviruses (ISFVs), are a historically understudied group of viruses due to their apathogenic nature in vertebrates. Aripo virus (ARPV) is a newly discovered ISFV isolated from *Psorophora albipes* mosquitoes in Trinidad. Unlike most ISFVs, ARPV is capable of vertebrate cell penetration and induction of an immune response in the absence of viral replication. Phylogenetically, ARPV also clusters more closely with pathogenic flaviviruses than previously characterized ISFVs. Here, we used ARPV as a vaccine vector to develop a pseudo-inactivated chimeric Zika virus (ZIKV) vaccine expressing ZIKV prM and E genes. The ARPV/ZIKV chimeric vaccine is highly safe by virtue of its complete replication deficiency in vertebrate cells, yet demonstrates immunogenicity superior to traditionally inactivated vaccines. Both ARPV and ARPV/ZIKV demonstrate no evidence of viral replication in mammalian cells *in vitro*, retaining this host-restriction throughout multiple blind serial passages. ARPV and ARPV/ZIKV also exhibit no pathogenicity in multiple *in vivo* murine models. Single-dose ARPV/ZIKV-vaccinated IFNAR$^{-/-}$ and C57BL/6 mice developed robust anti-ZIKV immune responses, producing antibodies in protective quantities as early as seven days post-vaccination. After lethal ZIKV challenge, ARPV/ZIKV-vaccinated mice were completely protected from viremia, weight loss, and mortality. A single dose of ARPV/ZIKV also prevented *in utero* ZIKV transmission in gravid IFNAR$^{-/-}$ mice. Vaccinated dams and their embryos exhibited no morbidity post-challenge, and no detectable ZIKV was present in placental, spleen, or brain tissues. Additionally, ARPV/ZIKV-inoculated C57BL/6 mice demonstrated robust T-cell responses with significant upregulation of T_H1, T_H2, and activated $CD4^+$ and $CD8^+$ T-cells, as well as key antiviral cytokines. Overall, the exceptional safety and efficacy of ARPV/ZIKV make it an ideal vaccine candidate and emphasize the potential of ARPV and other ISFVs for future flavivirus vaccine development.

1466

OBESE MICE HAVE A HIGHER MORTALITY RATE AND ALTERED IMMUNE RESPONSES FOLLOWING FLAVIVIRUS INFECTION IN COMPARISON TO WILD TYPE MICE

Elizabeth Geerling, Mariah Hassert, E Taylor Stone, Tara L. Steffen, Amelia K. Pinto

Saint Louis University, St. Louis, MO, United States

A rise in adiposity in the United States has resulted in more than 70% of adults being overweight or obese (National Center for Health Statistics). Globally, areas that have seen a dramatic rise in obesity have also seen a significant increase in emerging arboviral pathogens. Regions of South and Central America have particularly suffered from this "double disease burden" (World Health Organization). Studies done using influenza, hepatitis C, West Nile and dengue viruses have shown that excessive adiposity increases host susceptibility to viral infection and alters immune responses to vaccination. However, limited research has been done to identify mechanisms responsible for impaired immune function. Based on published studies and our preliminary data, we hypothesized that *obesity-associated chronic inflammation alters the immune system, leading to enhanced susceptibility to viral diseases and poor vaccine efficacy*. To study how obesity-associated immune inflammation impacts virus disease severity, we are using West Nile and Zika viruses in a murine model of obesity. By infecting regular chow-fed (wt) and high fat diet-induced obese (ob) mice, we compared virus-specific immune responses and viral loads at different time points post infection. Results from these studies indicate that female ob mice have a higher mortality rate and dysfunctional virus-specific T cell responses and lowered efficacy of neutralizing antibodies. These results demonstrate that obesity can lead to enhanced disease severity following flavivirus infection and may have long term consequences for the generation of protective immune responses to vaccination. We are currently conducting studies to determine if obesity leads to altered viral spread and tropism, as well as characterizing differences in immunological responses and protection from lethal challenge between ob and wt mice following vaccination.

1467

WEST NILE VIRUS GENOTYPE DISPLACEMENT IS DRIVEN BY INCREASED INFECTIVITY IN *CULEX* MOSQUITOES AND AVIAN TRANSMISSION EFFICIENCY

Sean M. Bialosuknia[1], Alan P. Dupuis II[1], Steven D. Zink[1], Cheri A. Koetzner[1], Joseph G. Maffei[1], Jennifer C. Owen[2], Hannah Landwerlen[2], Laura D. Kramer[1], Alexander T. Ciota[1]

[1]*NYS DOH, Wadsworth Center, Griffin Laboratory, Slingerlands, NY, United States, [2]Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI, United States*

Following its introduction into New York State (NYS) in 1999, *West Nile virus* (WNV; *Flavivirus*, *Flaviviridae*) underwent a rapid expansion throughout the U.S. and into Canada and Latin America. WNV has been characterized as being evolutionarily stable, with weak geographic structure, a dominance of purifying selection and limited adaptive change. While it is known that environmental factors influence regional and temporal fluctuations in WNV prevalence, less is known about the role of WNV genotype variability. Since the displacement of the NY99 genotype with WNV02, strains with increased transmissibility in *Culex* species mosquitoes have not been identified. A recent study identified

several novel positions with evidence of positive selection in the U.S., including positions identified in emergent genotypes in NY. One such genotype, designated NY10, is characterized by shared non-synonymous mutations in nonstructural genes. NY10 strains possess two shared amino acid substitutions, R1331K (NS2A) and I2513M (NS4B). We sequenced and analyzed 60 WNV isolates acquired from NY *Culex* mosquito pools obtained from 2013-2018. Our results indicate that the NY10 genotype has continued to displace historic genotypes, a process that began in 2010, concurrent with increased WNV activity. In addition, phenotypic studies demonstrate increased infectivity of NY10 strains in *Cx. pipiens* (χ^2, $p<0.05$) and one day longer viremia in *Turdus migratorius* when compared to WNV02, suggesting increased transmissibility likely facilitated displacement and contributed to increased WNV activity. Enhanced infectiousness and transmission in *Culex pipiens* suggest an order of magnitude lower threshold of infectiousness when considering the transmission from the avian host to mosquitoes. Additionally, the difference in viral kinetics between NY10 and WN02 suggests that birds infected with a NY10 strain may be infecting mosquitoes for longer. Together, these data provide novel insight into the evolutionary pressures on WNV and the need for continued genetic surveillance and characterization of emergent strains.

1468

STRUCTURE BASED ANALYSIS OF ANTIBODY BINDING TO FLAVIVIRUS E-DIMER AS MECHANISM OF POTENT NEUTRALIZATION

Cameron R. Adams[1], Huy Tu[2], Ellen Young[1], Sean Diehl[2], Ralph Baric[1], Aravinda de Silva[1], Premkumar Lakshmanane[1]

[1]*University of North Carolina, Chapel Hill, NC, United States,* [2]*University of Vermont, Burlington, VT, United States*

The flavivirus global health burden is exemplified by an estimated 396 million annual dengue virus (DENV) infections and the severe congenital disabilities caused by Zika virus (ZIKV). Primary infection by flaviviruses, including ZIKV, elicits a robust antibody response. However, only a small number of these antibodies can neutralize the infecting virus and confer long-term protection against re-infection. The flavivirus envelope (E) protein is the main target of neutralizing antibodies. E-protein is composed of three domains (EDI, EDII, and EDIII) and forms 90 antiparallel homodimers on the surface of the infectious virion. During viral entry and upon endosomal acidification, E-protein rearranges from dimers to trimers, involving large interdomain conformational changes. Most potently neutralizing human monoclonal antibodies (mAb) target quaternary epitopes that span across E-proteins and are hypothesized to lock the E-protein in the dimer conformation but this hypothesis lacks direct experimental evidence. We isolated a potently neutralizing mAb (G9E) (EC50 =1.3 ng/mL) from a patient with primary ZIKV infection. We determined the crystal structure of G9E in complex with recombinant ZIKV E-protein to 3.4 Å resolution. The structure revealed that G9E interaction is predominantly mediated by EDII of one E protein with noticeable peripheral contacts across E-homodimer. We designed structure-based G9E mAb variants with paratope changes to affect G9E binding across the E-dimer. The majority of G9E variants maintained similar binding affinity to ZIKV recombinant E-protein dimer when compared to G9E WT. G9E variants that altered one or two interactions across the homodimer showed only slight reduction in neutralization. However, a G9E variant (G9E-DNSK) that abrogated the majority of contact across the homodimer reduced in-vitro neutralization potency by >100-fold. These new structural insights into how antibodies mediate potent neutralization may lead to novel immune correlates of vaccine-mediated protection.

1469

ACTIVE CIRCULATION OF WEST NILE VIRUS AND SAINT LOUIS ENCEPHALITIS VIRUS IN TWO DENGUE ENDEMIC REGIONS OF COSTA RICA

Marta Piche-Ovares[1], Mario Romero-Vega[1], Diana Vargas-González[2], Daniel Barrantes-Murillo[1], Claudio Soto-Garita[1], Alejandro Alfaro-Alarcón[2], Carlos Jiménez-Sánchez[2], Eugenia Corrales-Aguilar[1]

[1]*Universidad de Costa Rica, San José, Costa Rica,* [2]*Universidad Nacional, Heredia, Costa Rica*

West Nile virus (WNV) and St. Louis Encephalitis Virus (SLEV) belongs to the family *Flaviviridae* and cause major zoonoses that have disseminated throughout the American continent. In Costa Rica, serological evidence of WNV circulation was found since 2004 in equine. Since then, clinical cases have been reported annually, especially in the lowlands of Guanacaste province. In the case of SLEV, the only previous record was found in Sloths. Two dengue and zika endemic regions of Costa Rica (Cuajiniquil and Talamanca) were selected for the detection of active WNV and SLEV transmission. In each location, 8 households were chosen, and blood samples were collected from people, horses, birds as well as mosquitoes in the rainy and dry season. RT-PCR for arbovirus ARN and PRNTs for serological evidence of infection were conducted. A total of 106 samples were obtained from equines, 33 from humans, 140 from birds, 39 from chickens, and 362 mosquitoes pools. At Cuajiniquil, serological evidence of WNV and SLEV was found in horses (24.4% WNV, 6.9% SLEV), humans (6.2%, 8.3%) chickens (6.2%, 3.4%), and birds (2.32%, 7.7%). Also, 5 seroconversions events were recorded: WNV (1 equine), SLEV (1 human), and DENV-1 (3 humans). In Talamanca, no evidence of WNV circulation was found, but evidence of SLEV was recorded in humans (17.6%), horses (60%), and birds (2.5%). Two seroconversions events were detected in human samples, 1 for DENV-1 and 1 for DENV-2. Furthermore, 12 organs of birds and 362 mosquito pools were analyzed by RT-PCR. No evidence of active infection was found in mosquitoes and bird samples. Of the mosquitoes sampled, *Culex quinquefasciatus*, one of the main vectors of these diseases, was the most abundant species in Talamanca and the second one in Cuajiniquil. Also, blood meals analyzed were done and signalizes that Culex species serve as bridge vectors between birds and mammals. Even though there is no actual information of the epidemiology of these viruses in those areas of Costa Rica, these data confirm the active circulation of SLEV at both sites and WNV in Cuajiniquil. Further studies have to be conducted to establish the genotypes that are circulating in the country.

1471

RELATEDNESS AND MUTATION SHAPE THE GENOMIC DIVERSITY OF RECURRENT *PLASMODIUM VIVAX* INFECTION

Aliou Dia[1], Catherine Jett[1], Simon G. Trevino[1], Cindy Chu[2], Kanlaya Sriprawat[1], Timothy Anderson[1], Francois Nosten[2], **Ian H. Cheeseman**[1]

[1]*Texas Biomedical Research Institute, San Antonio, TX, United States,* [2]*Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine Research Building, University of Oxford, Oxford, United Kingdom*

Infections with the malaria parasite *Plasmodium vivax* can recur after parasites have been cleared from the bloodstream. This may be due to either relapse from liver stage hypnozoites, recrudescence from blood stage parasites or reinfection by an infected mosquito bite. We explored genomic variation across recurrent *P. vivax* infections using a novel single cell sequencing protocol optimized for low parasitaemia infections, obtaining near-complete coverage of the parasite genome from individual cells. Genome sequences from 441 single parasite genomes from across 11 patients sampled during multiple febrile episodes confirm that in this setting recurrences are predominantly from identical or closely related genotypes and likely resulted from liver stage relapse or blood stage recrudescence. We combine conservative identification of *de novo* mutations arising within individual patients with phylogenetic tools to

explore the dynamics of recurrent infections at single genome resolution. We leverage this catalogue of *de novo* mutations to identify mutational hotspots in the parasite genome. We find recurrently hit gene families involved in antigenic variation and transcriptional regulation highlighting the capacity for intrahost evolution.

1472

A NOVEL AMPLICON DEEP SEQUENCING TOOL FOR STUDYING *PLASMODIUM VIVAX* INFECTIONS AT THE CLONAL LEVEL

Jason Rosado[1], Shazia Ruybal-Pesántez[2], Jacob Munro[2], Jiru Han[2], Zeinabou Traore[1], Ghania Boularias[1], Michael White[1], Melanie Bahlo[2], Alyssa Barry[3], Dionicia Gamboa[4], Ivo Mueller[1]

[1]*Institut Pasteur, Paris, France,* [2]*Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia,* [3]*Deakin University, Melbourne, Australia,* [4]*Universidad Peruana Cayetano Heredia, Lima, Peru*

Plasmodium vivax infections are characterized by recurrent bouts of bloodstage parasitaemia. To better understand the biology of *P. vivax* it is necessary to determine whether these are caused by a relapse or a new infection. Current genetic tools such as microsatellites do not always provide sufficient discriminatory resolution to address this question and whole-genome sequencing is not cost-effective nor easily transferable to malaria-endemic settings. In this context, amplicon deep sequencing (AmpSeq) allows detection of genetic variation at a lower cost by multiplexing many samples in a single sequencing run. We developed a novel AmpSeq tool targeting six short, but highly polymorphic SNP-rich regions, across six chromosomes. These loci were selected after screening 150bp windows of *P. vivax* whole genome sequences from MalariaGEN and ranking candidate loci based on expected heterozygosity (He). Validation was performed on field isolates from three endemic settings: Papua New Guinea (PNG) (n=8), Peru (n=12) and Solomon Islands (SI) (n=53). We validated this tool on both Illumina iSeq 100 and MiSeq platforms in paired-end mode (2x150bp). Microhaplotypes (MH) were called using the "HaplotypR" R package. Sequencing and MH calling were successfully executed in low-density samples (≥1.6parasites/μL) in both platforms. Minority clones (1% frequency) were detected with high sensitivity when coverage was ≥10,000 reads/marker. MHs had a median of 9 SNPs per marker (range=5–36) and the three most diverse markers per country were further analyzed to infer multiplicity of infection (MOI). As expected, multiclonal infections were more frequent in PNG (median MOI = 1.57) than Peru (median MOI = 1.28) and SI (median MOI = 1.28), consistent with the higher transmission in PNG. Analysis of laboratory clone mixtures is underway to estimate the clone detection limit. Further validation on longitudinal field samples from SI and Peru is ongoing to describe within-host clone dynamics in the context of recurrent infections. We anticipate this tool can be applied in a wide range of epidemiological studies and clinical trials.

1473

OVER 100 INDEPENDENT RECOMBINANT PROGENY FROM A NOVEL *PLASMODIUM FALCIPARUM* EXPERIMENTAL GENETIC CROSS

Ashley Michael Vaughan[1], Sudhir Kumar[1], Xue Li[2], Katrina Button-Simons[3], Spencer Kennedy[1], Ann Reyes[2], Mackenzie Sievert[3], Lisa Checkley[3], Meseret Haile[1], Abeer Sayeed[4], Katelyn Vendrely[3], Ian Cheeseman[2], Tim Anderson[2], Mike Ferdig[3]

[1]*Seattle Children's Research Institute, Seattle, WA, United States,* [2]*Texas Biomedical Research Institute, San Antonio, TX, United States,* [3]*University of Notre Dame, South Bend, IN, United States,* [4]*Texas Biomedical Research Institute, Seattle, TX, United States*

Experimental genetic crosses between clonal lines of *Plasmodium falciparum* require completion of the life cycle and have been used to isolate recombinant progeny for classical downstream phenotypic analysis and subsequent genetic linkage. This powerful approach has uncovered genes involved in antimalarial drug resistance, erythrocyte invasion, and mosquito immune invasion. However, the small number of unique recombinant progeny isolated in previous crosses using splenectomized chimpanzees (~30-50) limits the ability to accurately map polygenic traits. To overcome this, we have developed a human liver-chimeric mouse model (the FRG huHep mouse) to complete the liver stage-to-blood stage transition. We have recently carried out a genetic cross between an east African parasite, Mal31, a predominantly drug sensitive clone isolated from an individual with uncomplicated *P. falciparum* malaria in Chikhwawa, Malawi, and a southeast Asian parasite, KH004, a multi-drug resistant parasite clone isolated from an individual in Cambodia. We carried out this cross to investigate the genetic architecture that allows for the maintenance and spread of drug resistance to the frontline antimalarial artemisinin. Mature gametocytes from each clone were fed to mosquitoes, salivary gland sporozoites were isolated and then used to infect FRG huHep mice. After the liver stage-to-blood stage transition, ring stage parasites were adapted to *in vitro* culture and subsequently cloned. The power of this model was realized when we analyzed the 567 cloned samples: 144 were of mixed infection, 103 were parental and 320 were recombinant, of which 103 were unique. Of the 103 unique progeny, 95 had inherited their non-nuclear genomes (mitochondria and apicoplast) from the KH-004 parent. In addition, 58 progeny were represented only once, indicating that further cloning will isolate additional unique progeny. Allele frequencies across the genome of the unique progeny showed strong skews at chromosomes 7 (Mal31), 10 (KH-004) and 14 (Mal 31). Moving forward, the >100 progeny will be used in phenotypic studies to further understand the evolution of drug resistance.

1474

A METABOLOMICS APPROACH IDENTIFIES SPECIFIC BIOMARKERS OF DISEASE SEVERITY IN HUMAN CASES OF *PLASMODIUM KNOWLESI* MALARIA IN MALAYSIA

Regina Joice Cordy[1], Miriam Lachs[2], Mariko S. Peterson[2], Khamisah A. Kadir[3], ViLinh Tran[2], Karan Uppal[2], Dean P. Jones[2], Balbir Singh[3], Mary R. Galinski[2]

[1]*Wake Forest University, Winston-Salem, NC, United States,* [2]*Emory University, Atlanta, GA, United States,* [3]*Universiti Malaysia Sarawak, Kuching, Malaysia*

Malaria caused by the parasite *Plasmodium knowlesi* is a significant zoonotic disease and the most common form of malaria diagnosed in Malaysia. While rare as a parasite of humans worldwide, *P. knowlesi* malaria can cause severe and even fatal disease. In general with malaria and specifically with *P. knowlesi*, the various parasite and host factors that influence disease severity are complex and not well understood. To better understand factors that influence *P. knowlesi* disease severity, we performed metabolomics on 143 plasma samples acquired from people suffering from *P. knowlesi* who came into a district hospital in Kapit, Malaysia. We matched these with 111 uninfected individuals from the same geographical area. Metabolites were profiled in plasma samples using liquid chromatography-mass spectrometry (LC-MS) and untargeted high-resolution metabolomics (HRM) data analysis workflows. Metabolic pathways that were significantly different between infected and uninfected plasma include a range of amine, fatty acid and lipid pathways. This is in agreement with previous work suggesting that dynamic fluctuations occur in these groups of metabolites during malaria episodes. To further understand whether differences in metabolites were associated with differences in disease outcome, we further analyzed the *P. knowlesi* patient plasma metabolomes, and performed comparisons using clinical metadata such as parasitemia and hemoglobin. A subset of metabolites were found to vary significantly in these comparisons, demonstrating potential relationships between parasite load, anemia and systemic metabolic changes. Specifically, variation in kynurenine, a metabolic product of tryptophan breakdown with a role in immune tolerance, was observed across individuals with low versus high parasitemia. As kynurenine changes have also been noted in studies of *P. falciparum* and *P. vivax*, this finding supports the hypothesis that host control of *P. knowlesi* may involve common mechanisms as those seen with other human-infecting *Plasmodium* species.

1475

REGULATION OF *ANOPHELES* ADIPOKINETIC HORMONE SIGNALING IN MALARIA PARASITE SPOROGONY

Vincent O. Nyasembe, Timothy Hamerly, Prachi V. Khare, Borja López-Gutiérrez, Rhoel R. Dinglasan

University of Florida, Gainesville, FL, United States

Malaria, caused by *Plasmodium* parasites, kills close to half a million people and infects more than 200 million every year. An obligatory step in the complex life cycle of the malaria parasite is sporogony, which occurs only in female *Anopheles* mosquitoes. During this process, a single parasite undergoes multiple cellular divisions as a maturing oocyst over 12-17 days leading to the release of thousands of human-infective sporozoites. Consequently, the parasite relies on its *Anopheles* mosquito vector to provide essential nutrients to support oocyst development. Recent evidence suggests that malaria parasites manipulate the metabolic and immune pathways of its mosquito vectors to increase their transmission potential. We hypothesized that *Plasmodium falciparum* exploits the adipokinetic hormone (AKH) signaling pathway in its *Anopheles* vector to mobilize lipid reserves necessary for its metabolic needs and sporozoite development. To investigate this, we tested the effect of *P. falciparum* infection on the expression of AKH pathway genes in *Anopheles gambiae*. We then injected *P. falciparum*-infected *An. gambiae* with synthetic AKH and studied its effect on parasite development and sporozoite production. We further studied the effect of AKH gene knock-down and its subsequent rescue with synthetic AKH on midgut oocyst infection rates, intensity and diameter at eight days and salivary gland sporozoite counts at 14 days post infectious blood meal. Our findings show significant up-regulation of genes in the AKH pathway following *P. falciparum* infection. Perturbation of AKH levels using synthetic AKH and RNAi treatments significantly impacted sporozoite development. We discuss the implications of these finding in the context of parasite transmission potential and the design of novel malaria control strategies.

1477

USING PAI-1 TRANSGENIC MOSQUITOES TO TARGET FIBRINOLYSIS AND MALARIA TRANSMISSION

Tales V. Pascini[1], Yeong Je Jeong[1], Wei Huang[2], Zama R. Pala[1], Michael Wells[3], Juliana M. Sa[1], Christopher Kizito[2], Thiago Luiz Alves e Silva[1], Deborah Andrews[4], Thomas Wellems[1], Marcelo Jacobs-Lorena[2], Joel Vega-Rodriguez[1]

[1]*National Institute of Health (NIH), National Institute of Allergy and Infectious Diseases (NIAID), Rockville, MD, United States,* [2]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [3]*Johns Hopkins School of Medicine, Baltimore, MD, United States,* [4]*Johns Hopkins University School of Medicine, Baltimore, MD, United States*

Plasmodium parasites highjack the mammalian serine protease plasmin to facilitate migration through extracellular matrices and establish an infection in the mosquito and in the mammalian host. Plasminogen, the central protein of the fibrinolytic system, is an abundant zymogen in human blood and other tissues, which is activated into plasmin by the serine proteases tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA). Plasmin is primarily involved in the dissolution of fibrin clots and in tissue remodeling through the degradation of extracellular matrix proteins. Plasmin activation is fine regulated by plasminogen activator inhibitor 1 (PAI-1), a serine protease inhibitor that inactivates tPA and uPA. In order to block plasmin activation, parasite development and transmission, we engineered *Anopheles stephensi* transgenic mosquitoes that secrete human PAI-1 (huPAI-1) in the midgut lumen, in the saliva, or in both tissues. Tissue-specific expression of the huPAI-1 protein was shown for each transgenic line. Transmission-blocking assays with *Plasmodium berghei, P. falciparum, and P. vivax* show that expression of huPAI-1 in the saliva and/or the midgut lumen significantly inhibits oocyst intensity and prevalence. Importantly, the transmission-blocking effect of mosquito-expressed huPAI-1 was reversed by supplementing the infection with plasmin, showing that the reduction in infection is specific to the inhibition of plasminogen activation. Interestingly, expression of huPAI-1 in the salivary glands induce toxicity and structural deformation of the distal lateral lobes, resulting in reduced sporozoite invasion of the salivary gland. Finally, *P. berghei* transmission by mosquito bite on naïve mice was strongly impaired by the transgenic mosquitoes, resulting in a significant level of protection from malaria. In summary, we developed huPAI-1 transgenic mosquitoes that inhibit the parasite cycle in the mosquito and transmission to the mammalian host. Our results show the potential of targeting the fibrinolytic system to inhibit malaria transmission.

1478

DEVELOPMENT OF CONDITIONAL MALE-LETHAL SEXING *ANOPHELES STEPHENSI* LINE FOR PFSPZ PRODUCTION USING THE TET-ON SYSTEM

Ehud Inbar[1], Harrell II Robert[2], Donald Ward III[1], Robert Alford[2], Maryam Hosseini[1], Tao Li[1], Sumana Chakravarty[1], O'Brochta A. David[2], B. Kim Lee Sim[1], Peter F. Billingsley[1], Stephen L. Hoffman[1], **Abraham G. Eappen**[1]

[1]*Sanaria Inc., Rockville, MD, United States,* [2]*Insect Transformation Facility, Institute for Bioscience and Biotechnology Research, University of Maryland, Rockville, MD, United States*

Sanaria® PfSPZ Vaccine and PfSPZ-CVac have been highly protective in clinical trials in the US, Germany, and Africa. These vaccines are composed of aseptic, purified, cryopreserved *Plasmodium falciparum* (Pf) sporozoites (SPZ), and are manufactured using aseptically grown *Anopheles stephensi* female mosquitoes. A conditional sexing line of *A. stephensi,* in which superfluous male mosquitoes are removed from the system at the embryonic stage using conditional male specific lethal gene expression, will make the aseptic mosquito rearing process up to 100% more efficient at no additional cost. A conditional male lethal system in *A. stephensi* requires a lethal effector gene. While investigating the utility of a mosquito dominant-negative form of the immune deficiency (IMD) pathway transcription factor, Relish 2 (Rel2), we generated a dominant negative Rel2 (dnRel2) construct under control of *Drosophila melanogaster* UAS (upstream activation sequence), composed of the DNA-binding domain of Rel2 while the RNA-polymerase recruiting motif was deleted. Five transgenic *A. stephensi* lines, each driving GAL4 under the regulation of a different *A. stephensi* promoter, and an effector line carrying dnRel2 under the control of UAS were established. Crosses of the UAS-dnRel2 effector with any of the 5 GAL4 driver lines resulted in embryonic death of transgenic mosquitoes containing both the GAL4 driver and UAS-dnRel2 effector, making dnRel2 the lethal gene of choice. Taking advantage of the *A. stephensi* genome sequence, we identified 3 unique regions on the Y-chromosome that are targets for CRSPR/Cas9 -based gene insertion. *A. stephensi* bZIP1 gene is expressed in the early embryonic stage; therefore, the 5' region of this gene is being used to drive the expression of dnRel2 in males. We demonstrate that virgin and mated female *A. stephensi* have comparable PfSPZ infection intensities, suggesting that this approach will not negatively impact downstream vaccine manufacturing efficiency. We re now establishing a conditional male-lethal sexing *A. stephensi* line using the Tet-On system such that dnRel2 is expressed only in the presence of doxycycline.

1479

ASYMMETRIC MECHANISMS OF HYBRID MALE STERILITY IN RECIPROCAL CROSSES BETWEEN SPECIES OF THE *ANOPHELES GAMBIAE* COMPLEX

Jiangtao Liang, **Igor V. Sharakhov**

Virginia Tech, Blacksburg, VA, United States

Hybrid male sterility (HMS) contributes to speciation by restricting gene flow between related taxa. Detailed cytological characterizations of reproductive organs in hybrid mosquito males is important for identifying genes that regulate fertility of the disease vectors. To investigate possible cellular and molecular causes of HMS, we performed crosses between

closely related species of the *Anopheles gambiae* complex: *An. merus* with *An. gambiae* or *An. coluzzii*. We demonstrate that HMS in the African malaria mosquitoes involves two defects in the reciprocal crosses: a premeiotic arrest of germline stem cells in degenerate testes and a failure of the reductional meiotic division of primary spermatocytes in normal-like testes. The premeiotic arrest in degenerate testes of hybrids is accompanied by a strong suppression of meiotic and postmeiotic genes and by reduced size of male accessory glands (MAGs). Compared with pure species and hybrid males with normal-like testes, F1 males with degenerate reproductive organs display a shorter copulation time with females and they fail to produce mating plugs to induce female oviposition and monogamous behavior. This is despite the fact that degenerate MAGs in F1 males still express 20E hormone pathway genes. In normal-like testes of F1 hybrids, sex chromosomes are largely unpaired during meiotic prophase I and all chromosomes show various degrees of insufficient condensation. Instead of entering reductional division in meiosis I, primary spermatocytes prematurely undergo an equational mitotic division producing nonmotile diploid sperm. These results demonstrate that likely different sets of genes play roles in HMS in reciprocal inter-species crosses in the An. gambiae complex.

1481

POPULATION GENOMICS OF *ANOPHELES MINIMUS* IN CAMBODIA

Brandy St. Laurent[1], Nick Harding[2], Kirk Rockett[2], Eleanor Drury[1], Sonia Goncalves[1], Alistair Miles[2], Dominic Kwiatkowski[1]

[1]*Wellcome Sanger Institute, Cambridge, United Kingdom,* [2]*Big Data Institute, Oxford, United Kingdom*

Anopheles minimus is an important malaria vector throughout its wide geographic range across Southeast Asia. Genome sequencing could provide important insights into the unique malaria transmission dynamics in this region, where many vector species feed and rest outdoors, but as yet no population genomic studies have been performed on Southeast Asian anophelines. We describe results from a study using Illumina deep whole-genome sequences of 302 wild-caught *An. minimus* collected from three Cambodian provinces to examine the level of population structure and gene flow within this species over several years (2010, 2014-2016) and seasons. These specimens cluster into at least three distinct populations of *An. minimus s.s.* in Cambodia, with two populations overlapping geographically. We describe the underlying genetic diversity and divergence of these populations. We also investigated the genetic variation and recent selection in genes likely to be involved in insecticide resistance, including the voltage-gated sodium ion channel (Vgsc), known to confer resistance to DDT and pyrethroid insecticides. Cambodia is the focus of the emergence and spread of drug-resistant malaria parasites, so understanding the underlying genetic diversity and resilience of the vectors that transmit those parasites is key to implementing effective malaria control and elimination strategies. These data will be publicly available as part of the MalariaGEN Vector Observatory, an open access resource of genome sequence data.

1482

CONSTRUCTION AND ANALYSIS OF VISUALLY IMPAIRED *AEDES AEGYPTI* MUTANTS

Matthew R. Gregory, Andrew Cameron, Hannah Cunniff, Aidan Cook, Megan Decker, Brian Dineen, Daniel Guobadia, Patrick Kollman, Jeannie Nash, Caroline Seymour, Calla Sullivan, Joseph Y. Tang, Michelle A. Whaley, **Joseph E. O'Tousa**

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

Aedes aegypti mosquitoes act as vectors for numerous potentially fatal diseases including Zika, yellow fever, and dengue fever. Mosquitoes rely on visual, thermal, and olfactory cues to find hosts during disease transmission. Previous work in our laboratory revealed retinal specializations in *Aedes and Anopheline* mosquitoes that likely enhance the mosquito's ability to utilize visual information. Genetic mutants lacking critical components of the phototransduction pathway are valuable tools for determining how visual information contributes to the mosquito's behavioral responses. One key component is rhodopsin, responsible for photon capture and initiation of the visual response. Aaop1 is the rhodopsin found in the majority of the photoreceptors of the mosquito adult eye. This rhodopsin shows extensive movement into the photoreceptive membrane during the dusk period and away from these membranes following the dawn period. Both the prevalence of expression and the regulation of cellular location suggest this rhodopsin is providing visual capabilities during the mosquito's active periods at dawn and dusk. We used CRISPR gene editing to mutate the rhodopsin gene encoding Aaop1. We will present the molecular and cellular defects associated with the mutant alleles we have generated. We are developing host-seeking and other behavioral assays capable of visual capabilities mediated by the Aaop1 photoreceptors. The major larval rhodopsin of *Aedes* is Aaop3. Similar to the adult Aaop1 rhodopsin, Aaop3 rhodopsin undergoes dramatic light-mediated cellular movement. Accordingly, we created and characterized mutant alleles of Aaop3. We will present these results and describe the behavioral deficits associated with the Aaop3 mutant larvae.

1483

ONCHOCERCA VOLVULUS: DETECTION OF CIRCULATING CELL FREE DNA IN BODY FLUIDS THROUGH THE DETECTION OF A NOVEL HIGHLY REPETITIVE DNA SEQUENCE

Sasisekhar Bennuru, Chinweoke Osigwe, Frimpong Kodua, Eric Dahlstrom, Thomas Nutman

National Institutes of Health, Bethesda, MD, United States

The molecular diagnostic and xenomonitoring approach to the detection of *Onchocerca volvulus* (Ov) is based on the detection of the O-150 repeat. To identify an even more sensitive target, we used a Repeat Explorer-based pipeline using all available short read archived (SRA) raw sequence files to identify multiple extremely high copy repeat regions in the *O. volvulus* genome. Using custom primer-probe sets designed to amplify each of 16 different repeats we assessed their utility in distinguishing genomic DNA from O. volvulus from related filarial species (including *O. ochengi*, *Loa loa, Wuchereria bancrofti* and *Brugia malayi.* Of the 16, 2 of the targeting repeats termed Ov15R and Ov16R were found to be the most sensitive by qPCR (detection limit of 100 ag) with 100% specificity. Compared to using an O-150-based qPCR, we found that Ov15R and Ov16R was 10-64 times more sensitive. We next tested the utility of measuring Ov15R and Ov16R circulating cell-free DNA (ccfDNA) in *O. volvulus*-infected individuals. Using extraction of 1 ml of either serum or urine, we were able to detect Ov-ccfDNA in some but not all serum and urine samples. Interestingly, however, we could easily measure Ov-ccfDNA in the urine and serum of infected individuals within hours of treatment with DEC that peaked at day 5 post treatment and disappeared by 2 weeks. Currently, a more efficient method of Ov-ccFDNA extraction is being readied to allow for detection in all infected individuals' urine to allow for widescale non-invasive testing and monitoring of O. volvulus infection status.

1484

DESCRIPTION OF A *BRUGIA MALAYI* MUCIN, BM18019: A MODEL GLYCOPROTEIN TO UNDERSTAND CIRCULATING FILARIAL ANTIGENS

Marla Hertz, Amy Rush, Philip Budge

Washington University in Saint Louis, St Louis, MO, United States

The success of the Global Program for the Elimination of Lymphatic Filariasis depends on the accuracy of rapid diagnostic tests (RDTs) for LF. The LF-RDTs employ the monoclonal antibody AD12 to detect *Wuchereria bancrofti* circulating filarial antigen (Wb-CFA) via a specific carbohydrate epitope. This epitope is common in filarial nematodes, but little is known about its structure or function. Our lab identified an AD12-reactive glycoprotein, Bm18019, from *Brugia malayi* using in-gel proteomics of excretory/secretory (ES) products to serve as a model glycoprotein for structural and biochemical analysis. We generated a polyclonal antibody to

Bm18019 and found that, like the Wb-CFA, Bm18019 expresses PNGase F resistant AD12-reactive glycans. Bm18019 is a putative mucin sensitive to the mucinase O-sialoglycoprotein endopeptidase. The protein sequence consists of eleven repeat sequences, each 30-amino acids long and rich in serine and threonine. By comparing the predicted molecular weight to its native size by immunoblot, we estimate glycans account for half of the mass of the mature protein. Bm18019 has sex specific expression and is excreted by adult female worms while an immature, non-glycosylated form is secreted by microfilariae. Despite its similarities to Wb-CFA (AD12 glycosylation and secretion by adult worms), Bm18019 is not readily detected in human sera. Comparing Wb-CFA to Bm18019 may help explain why some filarial antigens are long-lived in human sera but others are not.

1485

ASSESSMENT OF SEROLOGICAL RESPONSES TO WUCHERERIA BANCROFTI AND ONCHOCERCA VOLVULUS DURING POST-TREATMENT SURVEILLANCE FOR LYMPHATIC FILARIASIS, PLATEAU STATE, NIGERIA

Rebecca Castor[1], Gregory S. Noland[2], Barminas Kahansim[3], Kenrick Anorue[3], Yohana Sambo[3], Abel Eigege[3], Solomon E. Adelamo[3], Bulus Mancha[3], Nils Pilotte[4], Steven A. Williams[4], Emily Griswold[2], Emmanuel S. Miri[3], Frank O. Richards Jr.[2]

[1]*Emory University, Atlanta, GA, United States,* [2]*The Carter Center, Atlanta, GA, United States,* [3]*The Carter Center, Jos, Nigeria,* [4]*Smith College, Northampton, MA, United States*

Nigeria bears the highest burden in Africa for lymphatic filariasis (LF) and onchocerciasis, two vector-transmitted filarial diseases caused by *Wuchereria bancrofti* and *Onchocerca volvulus*, respectively. This study compared results of an Ov16/Wb123 biplex rapid antibody test with laboratory-based ELISA for anti-Ov16 and anti-Wb123 antibodies and LF circulating filarial antigen (CFA) during post-treatment surveillance for LF. The study area was 3 local government areas of Plateau State, Nigeria that contained onchocerciasis meso-endemic and non-/hypo-endemic areas. From April to May 2016, finger-prick blood samples were collected in school-based Transmission Assessment Surveys of children approximately 6-7 years old and community-based household surveys of individuals >2 years. Rapid tests (Ov16/Wb123 biplex and immunochromatographic card test [ICT] for CFA) were conducted in the field; dried blood spots were collected for laboratory-based ELISA testing. A total of 6,854 individuals (median age: 7 years; range: 2-95) had matched demographic data and laboratory results. Overall prevalence of LF CFA, and Wb123 by ELISA and biplex were 0.13% (95% upper confidence limit [uCL]: 0.23), 1.75% (95% uCL: 2.03), and 0.13% (95% uCL: 0.23), respectively. CFA prevalence increased with age, while Wb123 prevalence by ELISA and biplex were highest in children 5-9 and in the oldest age group, respectively. Of the 9 samples positive for CFA, only one was Wb123 antibody biplex positive and none were Wb123 ELISA positive. None of the 120 individuals positive by Wb123 ELISA were positive by Wb123 biplex or for CFA. Overall prevalence of Ov16 by ELISA and biplex were 0.01% (95% uCL: 0.07) and 0.03% (95% uCL: 0.09), respectively. One of two Ov16 biplex positives was Ov16 ELISA positive. All Ov16-positive individuals were >50 years old. Compared to ELISA, the biplex rapid antibody test demonstrated good performance for Ov16, but poor performance for Wb123. Results indicate that transmission interruption of both diseases has been achieved in the study areas and provides important data on the age distribution of Wb123 and Ov16 in low transmission settings.

1486

A NEW ANTIBODY TEST FOR *WUCHERERIA BANCROFTI* INFECTION THAT IS USEFUL FOR ASSESSING THE IMPACT OF TREATMENT

Sarah E. Greene, Kurt Curtis, Peter U. Fischer, Gary J. Weil

Washington University in St Louis, Saint Louis, MO, United States

Lymphatic filariasis (LF) is a neglected tropical disease that impacts millions of people worldwide. Significant progress has been made towards eliminating LF by mass drug administration (MDA) to endemic populations. After MDA, microfilaria (MF) prevalence is typically reduced to very low levels, but filarial antigenemia often continues after MF clearance. Similarly, antibodies to filarial antigens such as Bm14 and Wb-123 often persist after effective treatment. The goal of this work was to develop an antibody test that would better reflect effective treatment then previously described antigen or antibody tests. We identified, cloned, expressed, and purified a novel *Wuchereria bancrofti* protein (provisional name WbN1) that is an ortholog of *Brugia malayi* BmR1, which is expressed by MF and used in the Brugia Rapid test. Antibodies to BmR1 are sensitive for the diagnosis of *Brugia* but not *W. bancrofti* infections. An IgG4 ELISA with WbN1 had ~90% sensitivity in patients with *W. bancrofti* microfilaremia and low-level cross-reactivity (<10%) for sera from patients with loiasis or onchocerciasis. We also assessed antibody levels before and after therapy with repeated, high doses of Ivermectin or DEC that cleared MF and filarial antigenemia in many patients. Antibodies to WbN1 fell by 96 weeks after either therapy. However, reductions in antibody levels relative to pretreatment (based on OD values) were greater for WbN1 than for Bm14. This was especially true in patients who were amicrofilaremic at 96 weeks, with OD reductions of 65% vs. 24%, respectively. Thus, WbN1 is a promising new antigen for serological detection of *W. bancrofti* infections that may be useful for assessing the impact of antifilarial treatment in individuals and populations.

1487

INTEGRATED SEROPREVALENCE ASSESSMENT OF *WUCHERERIA BANCROFTI* AND *ONCHOCERCA VOLVULUS* IN THREE DISTRICTS CO-ENDEMIC FOR LYMPHATIC FILARIASIS AND ONCHOCERCIASIS IN GAMBELLA REGION, ETHIOPIA

Mohammed Hassen[1], Aderajew Mohammed[1], Tekola Endeshaw[1], Tewodros Seid[1], Fikre Seife[2], Mossie Tamiru[2], Kadu Meribo[2], Emily Griswold[3], Moses Katabarwa[3], Frank Richards[3], Zerihun Tadesse[1]

[1]*The Carter Center, Addis Ababa, Ethiopia,* [2]*Federal Ministry of Health, Addis Ababa, Ethiopia,* [3]*The Carter Center, Atlanta, GA, United States*

Lymphatic filariasis (LF) and Onchocerciasis (River Blindness-RB) are among the neglected tropical diseases (NTD) targeted for elimination in Ethiopia. Many districts in Gambella Region, Ethiopia, are co-endemic for LF and RB and have been treated for over 6 years with ivermectin and albendazole through mass drug administration (MDA). Three co-endemic districts recently qualified for LF stop-MDA transmission assessment surveys (TAS-1). Ethiopia undertook an initiative to integrate serological surveys for RB stop-MDA evaluations into the TAS-1 in those districts. During May-June 2019, finger prick blood samples were collected from 6-7-year-old children in community-based samples determined using the LF TAS WHO survey sample builder. Blood from the same individuals was tested for LF circulating filarial antigen (CFA) in the field using Filarial Test Strips and collected onto Whatman #5 filter papers for laboratory testing for antibody to the RB Ov16 antigen. A total of 3,393 children from 150 villages in the three districts were tested; 1844 (54.3%) were male. All three districts had CFA results below the critical threshold for LF and passed TAS. In contrast, 40 children (1.2%) were positive for Ov16 antibody, well above the RB stop-MDA threshold of 0.1%. This integrated assessment allowed two decisions: a 'stop MDA decision' for LF and a 'continue MDA' decision for RB. Accordingly, the albendazole component of the MDA program was stopped but the ivermectin component was continued. We showed that a random sample for TAS can give important information about RB when results are positive, although the

usual approach to RB sampling is to sample from high risk villages near rivers ('first line' villages). Had the RB results been negative, additional investigation in older children and/or purposive sampling in high-risk areas would still have been needed. The TAS provided a valuable platform for assessing RB as well as LF.

1488

EFFICACY OF EMODEPSIDE AGAINST *ONCHOCERCA OCHENGI* IN NATURAL INFECTED CATTLE

Germanus S. Bah[1], Daniel Kulke[2], Nicolas H. Bayang[1], Sebastian Schneckener[3], Ralph Krebber[4], Henrietta F. Ngangyung[5], David D. Ekale[1], Youssouf M. Mfopit[1], John D. Graham-Brown[6], Vincent N. Tanya[1], Martin Glenschek-Sieberth[7], **Benjamin L. Makepeace**[6]

[1]*Institut de Recherche Agricole pour le Développement, Ngaoundéré, Cameroon,* [2]*Bayer Animal Health GmbH, Leverkusen, Germany,* [3]*Bayer AG, Leverkusen, Germany,* [4]*Bayer AG - Division Crop Science, Monheim, Germany,* [5]*Institut de Recherche Agricole pour le Développement, Mankon, Cameroon,* [6]*University of Liverpool, Liverpool, United Kingdom,* [7]*Bayer AG - Division Pharmaceuticals, Leverkusen, Germany*

Despite the efforts of several control programmes implemented during the past half-century, onchocerciasis continues to affect over 20 million people in sub-Saharan Africa. Mass drug administration with ivermectin has successfully prevented disease symptoms in onchocerciasis patients and has led to the cessation of transmission in selected foci. However, ivermectin lacks macrofilaricidal activity and has failed to break transmission in many areas with high levels of pre-control endemicity. Thus, a safe macrofilaricidal drug remains a research priority. Here, we evaluated the efficacy of emodepside, a cyclooctadepsipeptide compound licenced for the treatment of nematode infections in companion animals, against *Onchocerca ochengi* in naturally infected cattle. Pharmacokinetic studies in uninfected Holstein (*Bos taurus*) and Ngaoundéré Gudali cattle (*Bos indicus*) indicated that 0.15 mg/kg was equivalent to the human dose (10 mg) and 0.75 mg/kg was the maximum tolerated dose. Animals were randomised into six treatment groups (n = 7): Melarsomine (positive control) or vehicle only (negative control) alongside emodepside as a single humanised dose, multiple humanised dose (daily for 7 days), single maximum dose, or multiple maximum dose. Readouts included microfilarial density in skin and adult worm viability at regular intervals up until 18 months post-treatment. The single low dose had no discernible effect on either microfilariae or adult worms, whereas the multiple low dose and the single high dose suppressed microfilariae transiently. The multiple high dose induced a biphasic reduction in microfilariae, which were suppressed for three months before partial recrudescence and then full clearance in 4/7 animals by 18 months. Adult female worms from five cattle were dead or sterilised, but viable females were present in the remaining animals, in contrast with the 100% macrofilaricidal activity observed in the melarsomine group. In conclusion, emodepside has microfilaricidal activity against *O. ochengi* at lower drug exposure, with slow-acting sterilising and partial macrofilaricidal effects at higher doses.

1489

SPATIAL TEMPORAL MODELING OF LINKED EPIDEMIOLOGICAL AND GENOMIC DATA OF CHADIAN GUINEA WORMS REVEALS PROGRAMMATIC RELEVANT TRANSMISSION CHARACTERISTICS

Jessica Ribado[1], Nancy Li[1], Elizabeth Thiele[2], James Cotton[3], Adam Weiss[4], Hubert Zirimwabagabo[4], Philippe Tchindebet Ouakou[5], Tchonfienet Moundai[5], Guillaume Chabot-Couture[1], Joshua L. Proctor[1]

[1]*Institute for Disease Modeling, Bellevue, WA, United States,* [2]*Vassar College, Poughkeepsie, NY, United States,* [3]*Wellcome Sanger Institute, Hinxton, United Kingdom,* [4]*The Carter Center, Atlanta, GA, United States,* [5]*Ministry of Public Health, N'Djamena, Chad*

Guinea worm (*Dracunculus medinensis)* was detected in Chad in 2010 after a 10-year absence, posing a significant challenge to the global eradication effort. Initiation of a village-based surveillance system in 2012 revealed a substantial number of dogs infected with Guinea worm, raising questions about paratenic hosts and cross-species transmission. In this work, we use genomic data collected from 371 worms linked with epidemiological data from 2015-2018 to investigate the modes of transmission between hosts and the geographic connectivity between genetically similar worm populations. We identified recurring genotypes of these worms using 86 variants found on three mitochondrial loci to create identifying barcodes. There are 35 unique worm barcodes. Nine of these barcodes are shared by humans and dogs across multiple years, suggesting the possibility of human-dog transmission cycles. Spatio-temporal modeling reveals genetically identical worms are more likely to be within approximately 50 kilometers of each other. Smoothed density kernels of common barcodes (10 barcodes with at least 10 worms in the sample population) shows that barcodes vary in their degree of spatial clustering, suggesting there may be different transmission constraints. We demonstrate that these relationships are robust by comparing to a geostatistical null hypothesis constructed using the sampling frame of the available data. Using spatio-temporal models, our work extends previous investigations by revealing a consistent geographic connectivity between sets of worms with identical genomic sequences. These results suggest that scaling up genomic surveillance for Guinea worm may provide additional value to programmatic decision-making in the coming years which are critical for global eradication efforts.

1491

TAILORING MALARIA ROUTINE ACTIVITIES WITHIN THE COVID-19 PANDEMIC: A RISK AND MITIGATION ASSESSMENT OF EIGHT COUNTRIES IN WEST AFRICA

Suzanne Van Hulle[1], Joseph Lewinski[1], Ghislain Nana[1], Chrestien Yameni[2], Dominque Guinot[3], Sylvia Mollet Sangare[1]

[1]*Catholic Relief Services, Baltimore, MD, United States,* [2]*Catholic Relief Services West Africa, Dakar, Senegal,* [3]*Catholic Relief Services Europe, Geneva, Switzerland*

The COVID-19 pandemic has required malaria programs to shift intervention strategies to ensure access to prevention commodities and routine case management are maintained. Catholic Relief Services (CRS) is the Global Fund (GF) malaria recipient in eight West African countries Benin, Nigeria, Niger, Mali, Sierra Leone, Guinea, Senegal, and the Gambia and has utilized WHO, GF, and others technical guidance to reorient programming activities to ensure the safety of healthcare workers, volunteers and recipients during COVID-19. A real-time risk and mitigation assessment were taken from country contingency plans to determine what changes to malaria programming should be made and the impact these have on the quality and uptake of services. Identifiable changes were made to both ITN and seasonal malaria chemoprevention (SMC) campaigns in six countries including conducting virtual microplanning and remote training, providing remote support to sub-national coordination team, carrying out day-of data validation prior to implementation, modifying data collection tools, switching from fixed point to door-to-door distribution, and contactless distribution of sulfadoxine-pyrimethamine and amodiaquine blister packs. Mitigating solutions for case management services included reemphasizing testing before treatment if possible but moving to presumptive treatment if needed, strengthening the referral networks to be used for suspected COVID-19 cases, and bolstering last-mile supply chains for health commodities in the event of global shortages. Changes to malaria SBC messaging were also made in all eight countries that supported adding COVID-19 signs and symptoms within IPC and mass SMS messaging, reinforcing social distancing during SBC activities, and altering community-based messaging to come through social media channels. This assessment provides a framework for countries to help determine key risks to malaria programming and solutions during the COVID-19 pandemic or other public health emergencies. Further review will be conducted to determine impact of COVID-19 on malaria incidence in these eight countries.

1492

OUTREACH TRAINING SUPPORTIVE SUPERVISION IMPROVED THE QUALITY OF MALARIA MICROSCOPY IN THE DEMOCRATIC REPUBLIC OF CONGO

Ange Landela[1], Séraphine Kutumbakana[1], Anselme Manyong[1], Lennie Kyomuhangi[2], Emanuel Yamo[2], Dieudonne M. Ngoyi[3], Aboubacar Sadou[4], Godefroid Tshiswaka[4], Lawrence Barat[5], Ricki Orford[5], Eric Mukomena[6], Pharath Lim[2]

[1]*PMI Impact Malaria, Kinshasa, Democratic Republic of the Congo,* [2]*PMI Impact Malaria, MCDI, Silver Spring, MD, United States,* [3]*Institut National de Recherche Biomédicale (INRB), Kinshasa, Democratic Republic of the Congo,* [4]*U.S. President's Malaria Initiative, Kinshasa, Democratic Republic of the Congo,* [5]*PMI Impact Malaria Project, PSI, Washington, DC, United States,* [6]*Programme National de Lutte contre le Paludisme (PNLP), Kinshasa, Democratic Republic of the Congo*

Malaria microscopy (MM) is the gold standard for malaria diagnosis. However, sustaining MM skills is a challenge. Outreach Training and Supportive Supervision (OTSS+) which involves onsite training, supervision and mentoring is effective in maintaining the quality of MM. The PMI Impact Malaria Project supported the National Malaria Control Program in the DRC to implement one round of OTSS+ in 109 health facilities (HF) in nine provinces from May to August 2019 aiming to improve the percentage attaining MM competence in parasite detection to greater than 90% at HF level. During the OTSS+ visit, supervisors interviewed laboratory staff, observed slide preparation, staining and slide reading and gave feedback to the HFs on their performance. The supervisors checked the availability of internal (IQA) and external quality assurance (EQA) systems, assessed stock levels of essential supplies required for MM, conducted proficiency testing and provided mentoring on the identified gaps. Paper checklists were used for data collection and data were entered into a database after completion of the round. Data were exported to Excel for cleaning and analysis. Out of 109 HFs visited 7% had essential supplies for MM, 41% participated in IQA and 97% in EQA proficiency testing. Among the HFs visited 81% met the minimum competence standard required for parasite detection but only 9% met the minimum standard in MM. 151 out of 339 (44.5%) microscopes were non-functioning. Recorded poor performance can be attributed primarily to the lack of quality reagents and quality microscopes in most HFs. Laboratory OTSS+ visits are important to evaluate the quality of MM at HFs to identify major problems and to take corrective actions to improve MM quality. Recommendations and next steps formulated after the visits were used to advocate to the head of HF to acquire microscopes and laboratory reagents, and to raise awareness among HF managers to set up IQA systems as part of good laboratory practice. Moving forward, the quality assurance mechanisms could prioritize increasing competence skills in parasite detection and onsite malaria microscopy training using proficiency testing.

1493

STRENGTHENING INTEGRATION OF MALARIA INTO REPRODUCTIVE, MATERNAL, NEONATAL, CHILD, ADOLESCENT HEALTH AND NUTRITION (MALARIA-RMNCAH+N) IN NIGERIA - MAJOR MILESTONES AND SUCCESSES

Victoria Erinle[1], Chinedu Chukwu[1], Isaac Adejo[1], Tom Hall[2], Olatayo Abikoye[3], Sonachi Ezeiru[3], Emmanuel Shekarau[4], Nnenna Ogbulafor[4], Bala Muhammed Audu[4], Olugbenga Mokuolu[4], Ojonye Ega[5], Kayode Afolabi[6], Olumuyiwa Ojo[7], Lynda Ozor[7]

[1]*Management Sciences for Health (MSH), Abuja, Nigeria,* [2]*Management Sciences for Health (MSH), Arlington, VA, United States,* [3]*Catholic Relief Services (CRS), Abuja, Nigeria,* [4]*National Malaria Elimination Program (NMEP), Abuja, Nigeria,* [5]*Ministry of Health, Abuja, Nigeria,* [6]*Reproductive Health Unit, Fed. Min. of Health, Abuja, Nigeria,* [7]*World Health Organization (WHO), Abuja, Nigeria*

Nigeria's malaria burden remains high despite significant progress in recent years with reduction in prevalence from 42% (2010) to 23% (2018 DHS). Women, newborns, children and adolescent girls, continue to be disproportionately affected contributing to the high maternal and under five mortality of 512/100,000 and 132/1,000 live births respectively (2018 DHS). Contributing factors include vertical implementation of interventions, and poor coordination and collaboration among government agencies and partners, translating into weak implementation of the RMNCAH+N continuum of care at facility and community levels. To address this integration challenge, Management Sciences for Health, in collaboration with the National Malaria Elimination Program, the Reproductive Health Division of the Federal Ministry of Health, Catholic Relief Services and WHO, with funding from the Global Fund Malaria Grant, conducted a desk review and gap analysis of the existing RMNCAH+N service delivery platforms. Key services assessed were antenatal care (ANC), expanded program on immunization, intercurrent use of malaria preventive and curative services and integrated community case management of malaria. Based on the findings, the project supported a participatory process for identifying bottlenecks at various levels. The result is the development of a national framework for Malaria-RMNCAH+N integration with the creation of a Core Technical Committee for Integrated Disease and Health Interventions and Malaria-RMNCAH Advisory Technical Working Group. After Minister of Health approval, the framework was rolled out to sub-national levels. Other milestones achieved include streamlined communication and training strategies and materials, which incorporate malaria messages and components to build health worker capacity on integrated management of childhood illnesses, program management and ANC. Systems have been put in place to monitor and evaluate the impact of these various approaches. We recommend that countries actively explore this approach with relevant adaptation to strengthen malaria-RMNCAH+N integration.

1494

USING A MODIFIED CHALLENGE MODEL TO IDENTIFY MALARIA DATA ISSUES & IMPROVE KEY PERFORMANCE INDICATORS IN LIBERIA

Eric Diboulo[1], D. Levi Hinneh[2], Victor S. Koko[2], O. Joseph Alade[2], Patrick Konwloh[3], Julius Gilayeneh[2], Oliver J. Pratt[2], Luke L. Bawo[3], Jessica Kafuko[4], Agneta Mbithi[5], Yazoumé Yé[6]

[1]*ICF International Inc., Monrovia, Liberia,* [2]*National Malaria Control Program (NMCP), Monrovia, Liberia,* [3]*Ministry of Health, Monrovia, Liberia,* [4]*U.S. President's Malaria Initiative (PMI), Monrovia, Liberia,* [5]*ICF International Inc., Nairobi, Kenya,* [6]*ICF International Inc., Rockville, MD, United States*

In Liberia, 15 county health teams (CHTs) used a modified "Challenge Model" (an analytical and planning tool) to review technical challenges and identify priorities for technical support in malaria. Priority areas identified included data quality, data analysis interpretation, and data use. In response, the National Malaria Control Program (NMCP) and MEASURE Evaluation developed a series of tools to improve data quality, promote data analysis and interpretation, and track the performance of key malaria indicators at health facilities (HF) and at district and county levels. The South Eastern A region of Liberia has three counties, 20 health districts, and 69 HFs. Twenty percent of HFs were randomly selected in six districts to receive the Malaria Wall Chart (MWC) and the staff was trained to use it. The MWC is a chart that allows plotting of the monthly malaria key indicators' performances. The districts also received electronic performance tracking tools for malaria data quality and indicators. The NMCP provided monthly mentoring to selected districts' staff via email and phone. After six months of implementation, a data quality assessment was conducted to compare the data quality in selected HFs and districts. Before implementing the MWC in the second quarter of 2019, 25% of HFs were submitting inconsistent malaria diagnostic data versus in the

fourth quarter when all data was consistent (Q4) (p<.05). Ten percent of HFs had submitted inconsistent malaria treatment data versus 0% in Q4 (p<.05). Regarding antenatal care data, 30% of HFs submitted inconsistent data in Q2 versus zero percent in Q4 (p<=.01). Similarly, the number of HFs submitting inconsistent data on long-lasting insecticidal nets went from 25% of HFs in Q2 to 0% Q4 (p<.05). Malaria indicator tracking tools were proven effective in improving data quality. Beyond the measures of timeliness and completeness, data also improved in consistency and accuracy in HFs and in districts that had implemented the tools. The tools also helped to promote data review and data use at HFs. If these tools are used consistently, they have the potential to significantly improve the quality of malaria data.

1496

EVIDENCE OF IMPROVED MALARIA CASE MANAGEMENT BY PRIVATE SECTOR PROVIDERS THROUGH THE PROVISION OF SUBSIDIZED RDTS IN MADAGASCAR

Bakoly Nirina Rahaivondrafahitra[1], Mickael Randriamanjaka[1], Mauricette Andriamananjara[2], Saraha Rabeherisoa[2], Jacky Raharinjatovo[1], Ilo Andriamanamihaja[1], Aarons Chea[3], Christopher Lourenco[4], Stephen Poyer[4]

[1]*Population Services International -Madagascar, Antananarivo, Madagascar,* [2]*National Malaria Control Program, Antananarivo, Madagascar,* [3]*Population Services International, Nairobi, Kenya,* [4]*Population Services International -Washington, Washington, DC, United States*

The private healthcare sector is the first recourse to treatment for a third of childhood fever cases in Madagascar. Historically, diagnostic test availability in private health facilities has remained under 50%, achieved through donor support. Between 2018 and 2021 the Global Fund procured 1,621,250 RDTs for distribution to private providers, supported by comprehensive training and supervision activities. We conducted pre- (Feb 2018) and post- (Mar 2020) client exit interviews at a random sample of private facilities receiving RDTs to evaluate provider case management performance. Data were collected during the peak malaria transmission season and took place over three days at each facility. Eligible clients were adults aged 18 years and above seeking treatment for fever for themselves or on behalf of someone else. Self-reported information was captured on counselling, diagnostic services and medications received. Chi-squared tests were performed in Stata 13.0 and analysis accounted for the clustered nature of the survey data. 1,049 eligible clients from 247 facilities were interviewed at baseline and 1,276 clients from 249 facilities were interviewed at endline. 121 facilities from the baseline round were unavailable at endline as they had stopped receiving RDTs from the project or closed; they were replaced with additional active facilities. The median number of eligible clients per facility was 4.2 (2018) and 5.1 (2020). Eligible clients were more likely to report receiving a malaria RDT in 2020 compared to 2018 (42.4% vs. 13.1%, p=0.001) and more likely to report a positive result for malaria in 2020 (37.7% vs. 23.4%, p=0.01). The percentage of positive patients who received an ACT nearly halved between the two rounds (58.0% in 2018 vs. 32.1% in 2020, p=0.01), a result linked to reported stockouts of the first-line ACT in Madagascar. Continued improvement in private sector case management through the provision of RDTs has been seen but work remains to be done to reach the national target of 100% cases tested. Strategies are particularly needed to motivate private providers to continue stocking and offering diagnostic tests for malaria.

1497

INHIBITION OF THE *PLASMODIUM FALCIPARUM* ACETYL-COA SYNTHETASE BY MULTIPLE CHEMOTYPES DISRUPTS PROTEIN ACETYLATION AND EPIGENETIC REGULATION IN BLOOD STAGE PARASITES

Robert L. Summers[1], Charisse F. Pasaje[2], Manu Vanaerschot[3], James M. Murithi[3], Madeline R. Luth[4], Justin T. Munro[5], Pamela Magistrado-Coxen[1], Emily F. Carpenter[6], Jade Bath[3], Joao P. Pisco[7], Avinash S. Punekar[7], Beatriz Baragaña[7], Ian H. Gilbert[7], Manuel Llinás[8], Sabine Ottilie[4], Elizabeth A. Winzeler[4], Marcus C. Lee[6], Jacquin C. Niles[2], David A. Fidock[9], Amanda K. Lukens[10], Dyann F. Wirth[1]

[1]*Department of Immunology & Infectious Diseases, Harvard T.H. Chan School of Public Health, Boston, MA, United States,* [2]*Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA, United States,* [3]*Dept. of Microbiology & Immunology, Columbia University Irving Medical Center, New York, NY, United States,* [4]*Department of Pediatrics, University of California San Diego School of Medicine, La Jolla, CA, United States,* [5]*Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA, United States,* [6]*Wellcome Sanger Institute, Hinxton, United Kingdom,* [7]*Wellcome Centre for Anti-Infectives Research, Drug Discovery Unit, Division of Biological Chemistry and Drug Discovery, University of Dundee, Dundee, United Kingdom,* [8]*Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA, United States,* [9]*Dept. of Microbiology & Immunology & Division of Infectious Diseases, Dept. of Medicine, Columbia University Irving Medical Center, New York, NY, United States,* [10]*Infectious Disease and Microbiome Program, Broad Institute, Cambridge, MA, United States*

Global malaria control and elimination efforts rely on new generations of compounds with novel modes of action against the malaria parasite. Recently, *in vitro* evolution experiments have identified mutations in the parasite's Acetyl-CoA Synthetase (PF3D7_0627800; PfAcAS) which confer resistance to structurally distinct compounds, including MMV084978 and MMV019721. Allelic exchange using the CRISPR/Cas9 system confirmed that the A597V or T648M mutations in PfAcAS phenocopied the resistance phenotype. Conditional knockdown using the Tet-DOZI-RNA-aptamer system demonstrated that PfAcAS is essential for parasite growth, and partial knockdown sensitized parasites to both compounds. MMV019721 and MMV084978 directly inhibited recombinant PfAcAS activity in a substrate-competitive manner, with Ki values of 73 and 369 nM respectively, and the A597V mutation reduced inhibitor affinities by more than 90-fold. Orthologues of PfAcAS in eukaryotes catalyze the formation of the central metabolite acetyl-CoA from acetate, coenzyme A and ATP, and participate in a range of essential processes including epigenetic regulation. Metabolomic analyses revealed that exposure of trophozoite parasites to PfAcAS inhibitors reduced cellular acetyl-CoA levels by ~4-fold. To further investigate the biological implications of PfAcAS inhibition, western blot analyses were conducted to examine histone acetylation changes following brief exposures to MMV019721, MMV084978 and other PfAcAS inhibitors. Significant reductions in the acetylation of H3 and H4 histones at the H3K9, H4K8 and H4ac4 sites were observed for all inhibitors in wildtype but not in resistant parasites (ANOVA, $p < 0.05$). Knockdown of PfAcAS expression by 70% also reduced acetylation of H4K8 and H4ac4 markers by 92% and 40% respectively (T-test $p < 0.05$). Together these findings suggest that PfAcAS may play a role in maintaining nucleo-cytosolic acetyl-CoA pools that are necessary for the epigenetic regulation of parasite gene expression. These findings identify PfAcAS as a promising drug target with inhibitors that exert their effects on the parasite by a unique mode of action.

1500

A FIRST IN HUMAN STUDY TO INVESTIGATE THE SAFETY, PHARMACOKINETICS AND ANTIMALARIAL ACTIVITY OF ZY-19489 IN THE INDUCED BLOOD STAGE *PLASMODIUM FALCIPARUM* MALARIA MODEL

Bridget E. Barber[1], Melissa L. Fernandez[1], Hardik B. Patel[2], Stephen Woolley[1], Azrin N. Abd-Rahman[1], Ilaria Di Resta[3], Aline Fuchs[3], Harilal V. Patel[2], Stephan Chalon[3], Parmar V. Deven[2], James S. McCarthy[4], Kevinkumar Kansagra[2]

[1]*QIMR Berghofer Medical Research Institute, Brisbane, Australia,* [2]*Cadila Healthcare Ltd, Ahmedabad, India,* [3]*Medicines for Malaria Venture, Geneva, Switzerland,* [4]*QIMR Berghofer Medical Research Institute, Brisbane, Malaysia*

ZY-19489 is a new antimalarial that is active *in vitro* against blood stages of *P. falciparum* and *P. vivax*. We conducted a phase 1a/1b study to evaluate the safety, tolerability, pharmacokinetics and antimalarial activity of a single oral dose of ZY-19489 in healthy volunteers. The induced blood-stage malaria (IBSM) model was used to evaluate anti-malarial activity. In the phase 1a study, escalating doses of 25 to 1500 mg were administered. Emerging pharmacokinetic (PK) and safety data were evaluated by a safety data review committee and informed dose selection of later cohorts. The PK profile up to 28 days after dosing was described using a 2-compartment model with zero-order absorption, linear elimination and lag time, and with a non-linear increase in exposure with dose. Median elimination half-life was 75 hrs (95% CI 65 - 85 hrs). Testing for a food effect with the 300 mg dose did not demonstrate differences in exposure between fasted and fed conditions. For the IBSM study, a 300mg dose was administered in the first cohort, followed by doses of 200mg and 900 mg in cohort 2. The 300 mg dose resulted in rapid parasite clearance with a median parasite clearance half-life of 5.8 hrs (95% CI: 5.2 - 9.0). Administration of 200 mg ZY-19489 resulted in similar parasite clearance kinetics. Median parasite clearance half-life was 5.4 hrs (95% CI: 4.7 - 20.1). Administration of 900 mg ZY-19489 resulted in complete clearance of parasitemia in 2 of 2 subjects, without any recrudescence. PK/PD modelling for the 200, 300 and 900 mg dose is underway to identify the minimum inhibitory concentration and to guide dose selection for future clinical development. Adverse events in this study were mostly mild to moderate and were all transient in nature. No serious or severe drug-related adverse events occurred. In summary, this study demonstrates that ZY-19489 has a positive benefit/risk profile and supports its further clinical development as a single dose treatment of malaria.

1501

EVALUATION OF THE EFFICACY AND SAFETY OF TAFENOQUINE CO-ADMINISTERED WITH DIHYDROARTEMISININ-PIPERAQUINE FOR THE RADICAL CURE (ANTI-RELAPSE) OF *PLASMODIUM VIVAX* MALARIA IN INDONESIA - INSPECTOR STUDY

J. Kevin Baird[1], Inge Sutanto[2], Amin Soebandrio[3], Lenny Ekawati[1], Rintis Noviyanti[3], Disala Fernando[4], Eve Cedar[5], Alessandro Berni[5], Katie Rolfe[5], Sion Jones[5], Stephan Duparc[6], Lionel Tan[5]

[1]*Eijkman Oxford Clinical Research Unit (EOCRU), Jakarta, Indonesia,* [2]*Faculty of Medicine, University of Indonesia, Jakarta, Indonesia,* [3]*Eijkman Institute for Molecular Biology (EIMB), Jakarta, Indonesia,* [4]*GlaxoSmithKline, Addenbrookes Hospital, Cambridge, United Kingdom,* [5]*GlaxoSmithKline, Stockley Park, Uxbridge, United Kingdom,* [6]*Medicines for Malaria Venture, Geneva, Switzerland*

Tafenoquine (TQ) is a single-dose, 8-aminoquinoline indicated for *Plasmodium vivax* radical-cure when co-administered with chloroquine (CQ). In Indonesia, dihydroartemisinin-piperaquine (DP) is recommended for blood-stage malaria due to widespread CQ resistance. We conducted a double-blind, double-dummy, placebo-controlled study of TQ co-administered with DP for radical-cure. 150 G6PD-normal subjects from the Indonesian army with microscopy-confirmed *P.vivax* were enrolled at 2 malaria-free sites in Java following deployment in Indonesian Papua. Subjects received open-label DP over 3 days and randomized (1:1:1) to supervised concurrent treatment with: TQ 300mg (single-dose) or primaquine (PQ) 15mg for 14 days or DP alone. Subjects were followed-up for 180 days. TQ+DP was not associated with clinically relevant reduction in relapse over 6 months. Kaplan-Meier relapse-free efficacy estimates (microbiological Intent-to-Treat population) at 6 months were 21% (95% CI: 11,34) for TQ+DP, 52% (95% CI: 37,65) for PQ+DP and 11% (95% CI: 4,22) for DP only. Hazard ratio of the risk of relapse versus DP alone (by Cox's proportional hazards model) was 0.44 (95% CI: 0.29, 0.69) for TQ+DP and 0.26 (95% CI: 0.16, 0.43) for PQ+DP. Adverse events were as expected given the known safety profiles of the individual drugs. Low anti-relapse efficacy was demonstrated with TQ+DP co-administration. These results are inconsistent with previous studies of TQ+CQ co-administration which showed superior anti-relapse efficacy than CQ alone and comparable efficacy to PQ+CQ. TQ pharmacokinetic (PK) data from this study are not yet available due to delays associated with Covid-19. However, a previous healthy volunteer study found no significant PK interaction between TQ and DP. The reasons for lack of efficacy of TQ+DP in this study are being explored.

1502

A RANDOMIZED, OPEN-LABEL, NON-COMPARATIVE, MULTICENTER STUDY TO ASSESS THE PHARMACOKINETICS, SAFETY, AND EFFICACY OF TAFENOQUINE IN THE TREATMENT OF PEDIATRIC SUBJECTS WITH *PLASMODIUM VIVAX* MALARIA (TEACH STUDY)

Ivan D. Velez[1], Hien Tran[2], Ana Martin[3], Hema Sharma[3], Vicki Rousell[3], Liz Hardaker[3], John Breton[3], Terry Ernest[4], Katie Rolfe[3], Maxine Taylor[4], Stephan Duparc[5], Justin Green[3], Navin Goyal[6], Lionel Tan[3]

[1]*University of Antioquia, Medellin- Antioquia, Colombia,* [2]*Oxford University Clinical Research Unit, Hospital for Tropical Diseases, Ho Chi Minh, Viet Nam,* [3]*GlaxoSmithKline, London, United Kingdom,* [4]*GlaxoSmithKline, Ware, United Kingdom,* [5]*Medicines for Malaria Venture, Geneva, Switzerland,* [6]*GlaxoSmithKline, Upper Providence, PA, United States*

Tafenoquine (TQ) is a single-dose, 8-aminoquinoline indicated for *Plasmodium vivax* radical cure in patients aged ≥16 years when co-administered with chloroquine (CQ). Children account for a significant proportion of *P. vivax* infections. Tafenoquine Exposure Assessment in CHildren (TEACH) study (NCT02563496) is an open-label pharmacokinetic (PK) bridging study evaluating PK of TQ (50 mg dispersible tablets & 150 mg adult tablets) in children with *P. vivax* to identify appropriate pediatric doses to match efficacious exposures observed in adults. Initial doses were determined using a population PK (Pop-PK) model with TQ PK data from >800 adult subjects to ensure pediatric TQ systemic exposure (area under the curve, $AUC_{0-\infty}$) was comparable to that observed with approved adult TQ 300mg single dose (target $AUC_{0-\infty}$ 96 µg.hour/mL) while accounting for size differences. The study primary endpoint was TQ $AUC_{0-\infty}$ by weight band from pediatric Pop-PK model. Secondary endpoints included safety and recurrence-free efficacy at 4 months. TEACH enrolled 60 patients in Vietnam (n=32) and Colombia (n=28), age range 2-15 years. Patients received CQ and a single, TQ dose adjusted according to 4 weight bands (≥5 to ≤10 kg; >10 to ≤20 kg; >20 to ≤35 kg; >35 kg). PK samples were collected at days 3, 15, 29 and 60 post-TQ dosing. Observed TQ exposure were analyzed using a Pop-PK model at 2 protocol defined interim analyses (n=16 & n=32) and dose amended per *a priori* criteria. Doses were adjusted in the lower 2 weight bands after the 1st interim analysis, but no dose adjustment was made after 2nd interim analysis as TQ exposures in all weight bands were close to target $AUC_{0-\infty}$. Final doses were ≥5 to ≤10 kg - 50 mg; >10 to ≤20 kg -100 mg; >20 to ≤35 kg - 200 mg; >35 kg - 300 mg. At both interim analyses there were no *P. vivax* recurrences and no new safety signals identified. PK bridging studies are routinely conducted to identify safe and efficacious dosing in children. Final PK, efficacy and safety analysis from TEACH is expected to enable appropriate dose recommendation in 4 weight bands for TQ co-administered with CQ for *P. vivax* radical cure in children <16 years.

1504

POSSIBLE DECREASE IN EFFICACY OF INTERCEPTOR G2 IN AREAS OF HIGHLY RESISTANCE *ANOPHELES GAMBIAE SENSU LATO* POPULATION IN BURKINA FASO

Antoine Sanou[1], Fatoumata Cissé[1], Moussa Wandaogo Guelbéogo[1], N'falé Sagnon[1], Phillip MacCall[2], Geraldine Foster[2], Hilary Ranson[2]

[1]*Centre National de Recherche et de Formation sur le Paludisme (CNRFP), Ouagadougou, Burkina Faso,* [2]*Liverpool School of Tropical medicine, Liverpool, United Kingdom*

Malaria vectors in Burkina Faso have one of the highest levels of pyrethroid resistance recorded. As many others sub-Saharan African countries Next Generation Net are being deployed to mitigate the effect of this resistance on the performance of standard pyrethroid Long-Lasting Insecticide-treated Nets (LLINs). During the last mass distribution campaign of LLINs, Interceptor G2 (IG2), a double-active ingredient net with 100mg/m^2 of alpha-cypermethrin and 200mg/m^2 of chlorfenapyr, was distributed in the Cascades Region. The current study aims to assess the effect of this net in experimental hut studies in the Cascades Region. Different types of LLINs including Permanet 2.0 (unwashed), Interceptor G2 (washed 20 times and unwashed) and Permanet 3.0 (containing the synergist PBO, washed 20 times and unwashed) were tested for their efficacy against *Anopheles gambiae sensu lato (s.l.)* in West African experimental hut. This assessment was carried out at two locations in southwestern Burkina Faso characterised by high insecticide resistance vectors and different vectors species composition. Vector mortality, blood-feeding inhibition and induced exophily rate were assessed. A total of 7196 mosquitoes were collected from which *An. gambiae s.l.* represented ~65%. The highest cumulative mortality rate 72 hours after exposure was observed with the washed-IG2 (~40%) followed by unwashed Permanet 3.0 (~35%) but the mortality of unwashed IG2 (~20%) did not differ significantly from the untreated net used as control (~15%). In addition, the IG2 nets only reduced blood-feeding rate by less than 20% for washed relative to untreated. IG2 nets performed less well than observed for other vector populations across Africa. The trials will be repeated in 2020 but these early data suggest there may already be some decreased of susceptibility to chlorfenapyr in Burkina *Anopheles gambiae s.l.* populations.

1505

ANALYSIS OF A LARGE DATABASE OF INTENSITY BIOASSAYS FOR PHENOTYPIC INSECTICIDE RESISTANCE MONITORING IN MALARIA VECTORS

Mara D. Kont[1], Ben Lambert[1], Jan Kolaczinski[2], Lucia Fernandez Montoya[2], Audrey Lenhart[3], Hilary Ranson[4], Rosemary S. Lees[4], Natalie Lissenden[4], Catherine L. Moyes[5], Penelope A. Hancock[5], Thomas S. Churcher[1]

[1]*Imperial College London, London, United Kingdom,* [2]*World Health Organization, Geneva, Switzerland,* [3]*CDC, Atlanta, GA, United States,* [4]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [5]*University of Oxford, Oxford, United Kingdom*

Over the past two decades, a clear rise in pyrethroid insecticide resistance has been documented in malaria vectors. Discriminating dose bioassays (DDB) estimate the prevalence of phenotypic resistance based on mosquito survival after exposure to insecticides. In high resistance regions, where most mosquitoes survive, DDBs cannot provide a sensitive quantification of differences in resistance between mosquito populations. To remedy this, intensity bioassays (IB) were introduced, in which *Anopheles* mosquitoes are exposed to a range of insecticide doses and, in each case, their mortality recorded. However, as the level of uncertainty in IB measurements has thus far not been characterised, the operational significance of differences between these measurements is not clear. Here, we analyse a new global database of over 1000 IBs. Our dataset was compiled by combining sources from the World Health Organization, President's Malaria Initiative, Liverpool School of Tropical Medicine and Oxford University and comprises of tests conducted on over 300,000 *Anopheles* mosquitoes between 1997 and 2019 in Africa, South-East Asia and the Eastern Mediterranean. Using hierarchical logistic regression modelling, we quantify the spatiotemporal changes in resistance intensity, with insecticide concentration and type. In addition, by comparing IB measurements with those from DDBs, we elucidate, for the first time, the additional information provided by IB experiments. These results indicated qualitatively similar levels of resistance to those estimated from DDB data, although there were often substantial quantitative differences. In contrast to DDB data, no significant and persistent trends over time were determined from analysing IB data: this held at the various intensity concentrations examined. Temperature and humidity were often found to affect the level of measured resistance in IBs. Overall, our results indicate the large variation inherent in IBs and the challenge presented in analysing them. Our work also suggests that naive comparison of data from few experiments is unlikely to yield operationally robust insights.

1507

EVALUATION OF PARTIAL INDOOR RESIDUAL SPRAYING: AN EFFECTIVE AND COST SAVING POTENTIAL ALTERNATIVE TO CONVENTIONAL SPRAYING FOR MALARIA VECTOR CONTROL

Sylvester Coleman[1], Yemane Yihdego[2], Dereje Dengela[2], Richard Oxborough[2], Ben Johns[2], Lilly Siems[2], Bradford Lucas[2], Samuel Dadzie[3], Frank Gyamfi[1], Edem Obum[1], Louisa Antwi-Agyei[1], Lena Kolyada[1], Jon Eric Tongren[4], Sixte Zigirumugabe[4], Dominic Dery[4], Christen Fornadel[5], Kristen George[5], Allison Belemvire[5], Jennifer Armistead[5], Jenny Carlson[5], Seth Irish[6], Aklilu Seyoum[2]

[1]*PMI VectorLink Project, Abt Associates, Plot 11 Waterson Road, Tamale, Ghana,* [2]*PMI VectorLink Project, Abt Associates, 6130 Executive Blvd, Rockville, 20852, MD, United States,* [3]*Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana,* [4]*U.S. President's Malaria Initiative, U.S. Agency for International Development, Accra, Ghana,* [5]*U.S. President's Malaria Initiative, U.S. Agency for International Development, Washington, DC, United States,* [6]*U.S. President's Malaria Initiative, Centers for Disease Control and Prevention, Atlanta, GA, United States*

Widespread pyrethroid resistance has necessitated the use of expensive insecticides for malaria vector control using indoor residual spraying (IRS). We evaluated whether partial spraying of houses, could potentially reduce costs without compromising the efficacy of IRS. In an initial experimental hut trial in northern Ghana, partial spraying of the top (IRR 0.89, p = 0.13) or bottom (IRR 0.90, p = 0.15) half of walls and the ceiling was not significantly less effective to full spraying with pirimiphos-methyl, as measured by mortality of *An. gambiae* s.l. A small-scale pilot was then conducted to assess the effectiveness, feasibility and cost implications of partial spraying of upper walls and ceiling as compared to full spraying in three communities in northern Ghana. The mean human biting rate of *An. gambiae* s.l. in both fully sprayed (6.42 bites/person/night (b/p/n)) and partially sprayed (9.64 b/p/n) sites was significantly lower (p<0.001) than in the control sites (19.8 b/p/n), but the difference between partially and fully sprayed areas was not significant (p=0.513). The post-spray mean parity rates of *An. gambiae* s.l. in both fully (39%) and partially sprayed communities (45%) were significantly lower (p<0.0001) than the control sites (66%), but the difference between partially and fully sprayed areas was not significant. The entomological inoculation rate over the 8-month study period was highest in the control site, 168 infective bites/person/8 months (ib/p/8mo), followed by the partially (33ib/p/8mo) and fully (25ib/p/8mo) sprayed sites. Partial spraying resulted in 33% savings on insecticide and operational costs that when extrapolated to the larger IRS campaign would have allowed for an estimated $496,426 in savings and spraying of an additional 36,000 structures. Among the communities interviewed, 40% were not willing to accept partial spraying of their homes, indicating that robust community sensitization may be required to scale up. These findings suggest that partial spraying is an effective and cost saving approach that could potentially be adopted to increase implementation of this key malaria control intervention.

1508

ASSESSMENT OF THE RESIDUAL EFFECTIVENESS OF CLOTHIANIDIN FOR THE CONTROL OF PYRETHROID RESISTANT MALARIA VECTORS IN NORTH WESTERN LAKE ZONE REGIONS IN TANZANIA

Karen Nelwin Zablon[1], Charles Kakilla[1], Jackline Martine[1], Eric Lyimo[1], Coleman Kishamawe[1], Doris Mangalu[1], Lucy Bernard[1], Safari M. Kinung'hi[1], Chonge Kitojo[2], Naomi Serbantez[2], Eric Reaves[3], Adeline Chan[3], Charles D. Mwalimu[4], Ssanyu Nyinondi[5], Bilali Kabula[5], Alphaxard Manjurano[1]

[1]*National Institute for Medical Research (NIMR), Mwanza Centre, United Republic of Tanzania,* [2]*US President's Malaria Initiative, United States Agency for International Development, Dar es Salaam, United Republic of Tanzania,* [3]*Centres for Disease Control and Prevention (CDC), Atlanta, GA, United States,* [4]*National Malaria Control Program, Ministry of Health, Community Development, Gender, Elderly and Children, Dodoma, United Republic of Tanzania,* [5]*RTI International, Dar es Salaam, United Republic of Tanzania*

Entomological surveillance was carried out in seven indoor residual spraying (IRS) and four non-IRS districts of the Lake Zone to determine the impact of IRS with clothianidin (SumiShield 50WG) from October 2018 to September 2019.Cone and fumigant bioassays were conducted monthly with laboratory susceptible *Anopheles gambiae* s.s. "Kisumu strain" as per World Health Organization (WHO) protocol. Sprayed households with different wall surfaces (i.e. cement, burnt brick, painted, mud and whitewash) were randomly selected for bioassay. Additionally, CDC-light traps, claypots, Prokopack aspirators and CDC-light traps with collection bottle rotators were used for monthly monitoring of behavior and dynamics of local malaria vectors. Clothianidin 50WG showed mortality rates of more than 80% WHO bio-efficacy threshold on all wall surfaces tested with susceptible *An. gambiae* s.s. over a period of 10 months. A total of 14,035 female *Anopheles* were collected and morphologically identified as *An. gambiae* s.l. (n=9,183; 65.4%), *An. funestus* s.l. (n=2,597; 18.5%), *An. coustani* (n=1,896; 13.5%), *An. pharoensis* (n=217; 1.5%) and *An. rufipes* (n=142; 1%). *An. gambiae* s.l. was the most abundant vector species sampled by all collection methods in IRS districts. Speciation by PCR (n=4,373) showed the local vector population to be predominantly *An. arabiensis* (53%), *An. funestus* s.s. (27.1%), *An. gambiae* s.s. (13.9%) and *An. parensis* (2.3%). Mean sporozoite rate was noticeably lower at 1.4% (95% CI: 1.0-1.9) in the IRS sites compared to 2.3% (95% CI: 1.6-3.2) in non-IRS sites hence decrease in indoor biting rates in sprayed sites post IRS. There was more indoor than outdoor biting in IRS areas. Clothianidin 50WG showed very good and lasting efficacy for IRS in the area where *Anopheles* mosquitoes are resistant pyrethroids. IRS with clothianidin has proved effective in controlling malaria transmission by *Anopheles* mosquitoes. Simultaneous application of IRS with outdoor vector control tools is likely to effectively reduce malaria transmission.

1509

USE OF COLORIMETRIC TESTS AND HPLC-PDA TO DETERMINE THE AMOUNT OF INSECTICIDES MOSQUITOES PICK UP FROM TREATED BED NETS

Mojca Kristan, Jo Lines, Harparkash Kaur

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

Insecticides used in vector control mainly work when vectors come into contact with treated surfaces, yet little is known about the amount adhering to malaria vectors. Measuring the amount is not only relevant for determining the doses that are lethal to the mosquito and optimising vector control tools but is also necessary to help understand how insecticides influence vector competence as they might affect sporogony and mosquito microbiota. Three to five days old non-blood fed female *Anopheles coluzzii* mosquitoes (N'gousso strain) were exposed to a long-lasting insecticidal net (PermaNet 2.0), using a wire ball frame. The amount of insecticide that adheres to mosquitoes from the net during different exposure times (0.5-3 min) was measured using high performance liquid chromatography-photodiode array method (HPLC-PDA). A colorimetric test was then used to visually detect the amount of deltamethrin on mosquitoes following exposure to the net. The resulting depth of colour was also recorded with a mobile phone to explore whether such images can be used to quantify the results in the field, in the absence of technologically sophisticated equipment. A colorimetric test, designed to detect the type 2 pyrethroids on nets and sprayed walls, was successfully used for the first time to detect deltamethrin on mosquitoes following exposure to the net. The confirmatory HPLC-PDA analysis determined that after 2 min exposure up to 12 ng of deltamethrin adhered to mosquitoes following exposure to PermaNet 2.0 (*mean = 5.2 ng/mosquito, SE = 1.9*) and that the final dose depends on the length of exposure time. Digital pictures can also be used to quantify the results and detect minute differences between samples. This study demonstrated the potential of a screening (type 2 pyrethroid colorimetric test) and a confirmatory test (HPLC-PDA) to determine the amount of insecticide that mosquitoes pick up on contact with treated surfaces, with implications for detection of specific active insecticide ingredients that cause the greatest mosquito mortality in circumstances where mixtures of insecticides may be used to maximise effectiveness of interventions.

1511

REVISITING DENSITY DEPENDENCE IN SCHISTOSOMES USING SIBSHIP RECONSTRUCTION

M. Inês Neves, Joanne P. Webster, Martin Walker

Royal Veterinary College, University of London, Hatfield, United Kingdom

The stability of parasite populations is regulated by density-dependent processes occurring at different stages of their life cycle. In dioecious helminth infections, density-dependent fecundity describes the reduction in egg production by female worms in high worm burden within-host environments. For human schistosomiasis, investigating density-dependent fecundity is hampered by the inaccessibility of adult worms within hosts, due to the intravascular location of the parasite. Whether egg production is regulated in a density-dependent manner is key to interpreting routine egg count data and to predicting the response of schistosome populations to perturbation by intervention. However, current understanding of this fundamental population process is limited to data collected from two autopsy studies conducted over 40 years ago, with subsequent analyses having reached conflicting conclusions. Sibship reconstruction is a branch of parentage analysis which can be used to estimate the number of parents/adult worms in individual human hosts from molecular data derived from the accessible transmission stages/offspring of schistosomes. In combination with egg count data, this provides a novel means to identify density-dependent fecundity, albeit requiring robust statistical methodologies to account for the bias and uncertainty of worm burden estimates, which depend on the number of offspring sampled. We illustrate this approach using a recent multiplexed microsatellite dataset derived from *Schistosoma haematobium* miracidia hatched from infected urine samples of children undergoing preventive chemotherapy in Zanzibar. We found a non-proportional relationship between *Schistosoma haematobium* egg counts and inferred numbers of female worms, indicating that egg production is suppressed in individuals with higher worm burdens, suggesting density-dependent fecundity. We discuss the public health implications of our findings, including for policy decisions informed by the modelled transmission dynamics of schistosomes and for the interpretation of egg count data collected during monitoring and evaluation activities.

1512

MISMATCHES IN THERMAL OPTIMA OF *SCHISTOSOMA MANSONI* AND *BIOMPHALARIA* SPP. LIFE-HISTORY TRAITS SHIFT THE THERMAL OPTIMUM OF HUMAN SCHISTOSOMIASIS TRANSMISSION UNDER DIFFERENT INTERVENTION SCENARIOS

Karena H. Nguyen[1], Philipp H. Boersch-Supan[2], Valerie J. Harwood[3], Jason R. Rohr[4]

[1]*Emory University, Atlanta, GA, United States,* [2]*British Trust for Ornithology, Thetford, United Kingdom,* [3]*University of South Florida, Tampa, FL, United States,* [4]*University of Notre Dame, Notre Dame, IN, United States*

Disease transmission between ectothermic parasites and their intermediate hosts depends on environmental conditions. Global climate change is therefore expected to influence host-parasite dynamics, but predicting these effects is predicated on measuring organisms' responses to temperature. We measured the thermal sensitivity of two life-history traits which have rarely been addressed, miracidial hatching success and cercarial emergence of *Schistosoma mansoni*. We then synthesized these life-history traits and experimentally determined thermal performance curves related miracidial, cercarial, and *Biomphalaria* spp. (i.e., snail) life-history traits to parameterize a mathematical model for schistosomiasis transmission based on R_0, which estimates the number of secondary infections given one infected human. We used the parameterized model to predict temperature-dependent risk of schistosomiasis transmission under three distinct intervention scenarios: anthelminthic drug treatment of humans, molluscicide application, and cercarial control. We found that miracidial hatching success increased linearly up to 37°C while cercarial emergence was greatest at 26°C. Other parasite and snail life-history traits had temperature optima between 13.7 and 22.1°C, resulting in a thermal optimum (T_{opt}) for R_0 at 21.7°C. Modeled transmission rates decreased in response to each intervention strategy, but targeting snail populations had the greatest effect on decreasing overall transmission as well as shifting the T_{opt} for R_0 by as much as 1.25°C warmer. Additional analyses revealed that additional mortality from molluscicide application exceeded natural and infection-related snail mortality, which then diminished the importance of the latter two life-history traits (which had cooler T_{opts}) and drove the overall T_{opt} for transmission to higher temperatures. Our results suggest that the effectiveness of control strategies for vector-borne diseases or diseases with environmental parasitic life stages could be altered if the T_{opt} for transmission is shifted closer to or farther away from temperature regimes in endemic regions.

1513

DYNAMICS OF PARASITE AGGREGATION UNDER INTENSE CONTROL EFFORTS: INSIGHTS FROM THE ZANZIBAR ELIMINATION OF SCHISTOSOMIASIS TRANSMISSION (ZEST) STUDY

Christopher M. Hoover[1], Stefanie Knopp[2], Alan Hubbard[1], Joseph A. Lewnard[1], Giulio A. de Leo[3], Susanne H. Sokolow[3], Fatma Kabole[4], David Rollinson[5], Justin V. Remais[1]

[1]*UC Berkeley, Berkeley, CA, United States,* [2]*Swiss Tropical and Public Health Institute, Basel, Switzerland,* [3]*Stanford University, Stanford, CA, United States,* [4]*Neglected Disease Program, Zanzibar Ministry of Health, Unguja, United Republic of Tanzania,* [5]*Natural History Museum, London, United Kingdom*

The aggregation of helminthic parasites has important implications for the transmission of neglected tropical diseases including schistosomiasis, lymphatic filariasis, and onchocerciasis. Only mated worm pairs contribute to transmission, and the mating frequency of worms is heavily influenced by their aggregation amongst definitive human hosts. Here we use data from the Zanzibar Elimination of Schistosomiasis Transmission (ZEST) study to determine how aggregation changes in response to control efforts such as mass drug administration (MDA). We first quantify aggregation via maximum likelihood estimation of the dispersion parameter, k, by fitting negative binomial distributions to egg intensity data collected from children enrolled in the study across 90 small administrative units called shehias from 2012-2017. We then use generalized estimating equations to estimate changes in k as a function of mean parasite burden. We find a -0.012 (95%CI -0.020 - -0.009) reduction in the dispersion parameter, implying increasing aggregation, associated with an interquartile range decrease in the mean shehia egg burden. In addition, we derive a novel mating probability function based on the mean worm burden and dispersion parameter that relaxes the common assumption that male and female parasites are distributed together. We incorporate this function into estimation of the mean parasite burden across all shehia-years in ZEST and find that the prevalence of single-sex infections is likely higher than previously recognized. These results suggest that identifying and treating infected individuals that sustain transmission may become increasingly difficult approaching elimination. The high apparent prevalence of single-sex infections may also serve as a silent reservoir of infection. Individuals with single sex infections can revert to patent, egg-shedding infections after acquiring a single parasite of the opposite sex, potentially contributing to rebounds in infection if control efforts are stopped prior to full elimination.

1514

SCHISTOSOMA MANSONI TRANSMISSION IN UGANDAN HOTSPOT AREAS. WHO IS REINFECTING WHOM?

Christina L. Faust[1], Olivia Ericsson[2], Moses Arinaitwe[3], Moses Adriko[3], Andrina Nankasi[3], Fred Besigye[3], Aaron Atuhaire[3], Edridah M. Tukahebwa[3], **Poppy HL Lamberton**[2]

[1]*Penn State University, State College, PA, United States,* [2]*University of Glasgow, Glasgow, United Kingdom,* [3]*Vector Control Division, Ministry of Health, Kampala, Uganda*

Over 240 million people are infected with schistosomiasis. The World Health Organization recommends mass drug administration (MDA) to control the disease, primarily focusing on school-aged children (SAC). Uganda began their MDA programme in 2003, but in some villages we continue to detect high levels of infection. Here we combine parasite molecular, population epidemiology and travel survey data across the entire community to ascertain what contributions different age groups have in driving transmission. We conducted a two year longitudinal survey of 595 individuals aged 9 months to 80 years residing in Bugoto, Mayuge District, Uganda, a high endemic *Schistosoma mansoni* community. Infection intensities, measured by three days of duplicate Kato-Katz, were highest in young adults (15-29 years old) at baseline. Five months after praziquantel treatment infection intensities were significantly lower in pre-SAC (<6 years old) and adults than at baseline, whereas intensities had returned to pre-treatment levels in SAC. Genetic data from mircadia collected at the same timepoints reveal significant population structure within the community. While there is parasite gene flow between all age groups, adults have a higher diversity and distinct parasites that reflect their travel history to other endemic areas. Although reinfection rates are much lower, genetic diversity after treatment suggest that adults serve as a common source for parasites across the population. Overall, our results highlight that improving drug coverage within adults, particularly young adults, will provide benefits to reduce overall prevalence and parasite genetic diversity in persistent hotspots. Additional interventions that reduce lake water contact and improve knowledge of schistosomiasis risk across the community, particularly in young adults and long-term residents, may be a cost-effective way to achieve progress in these hotspots.

1515

SCHISTOSOMA MANSONI INFECTION IN A HARD TO REACH DISTRICT OF MADAGASCAR FOLLOWING FOUR ROUNDS OF MASS DRUG ADMINISTRATION: RESULTS FROM REPEATED ANNUAL CROSS-SECTIONAL STUDIES

Stephen Spencer[1], James Penney[1], Hannah Russell[1], Cortland Linder[1], Caitlin Sheehy[1], Kate Hyde[1], J. R. Stothard[2], Amaya Bustinduy[3], Sheena Cruickshank[1], Emmanuel Andriamasy[4], Alain Rahetilahy[5]

[1]*University of Manchester, Manchester, United Kingdom,* [2]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [3]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [4]*Faculté de Médecine, Université d'Antananarivo, Antananarivo, Madagascar,* [5]*Ministère de la Santé Publique, Antananarivo, Madagascar*

There is a huge burden of disease due to *Schistosoma mansoni* in eastern Madagascar where the prevalence was 94% in 2015. Madagascar Medical Expeditions (MADEX) is a voluntary research organisation set up by students from the University of Manchester to tackle schistosomiasis in this region in collaboration with the Madagascar Ministry of Health. Between 2015 and 2019 we have investigated schistosomiasis prevalence and morbidity in school-age children in response to annual mass drug administration (MDA). Five repeated annual cross-sectional surveys were carried out in the Marolambo district, Madagascar. Prevalence of *S. mansoni* was determined by Kato-Katz microscopy and circulating cathodic antigen (CCA). Eggs per gram of stool (epg) of *S. mansoni* were calculated. Prevalence of *S. mansoni* by urine-CCA decreased from 94% (356/379) in 2015 to 86% (314/365) in 2019 ($p<0.0001$) and prevalence by Kato-Katz decreased from 74% (215/291) to 59% (204/345; $p<0.0001$). Prevalence of heavy infections (>400 epg) declined from 24% (69/291) in 2015 to 10% (34/345) in 2019 ($p<0.0001$) while moderate infections reduced from 23% (67/291) to 16% (55/345); $p=0.002$), and light infections remained unchanged (27% (79/291) to 33% (115/345); $p=0.124$). Despite five years of annual MDA of praziquantel in this region, intestinal schistosomiasis remains a significant public health challenge. Whist there has been general shift in the intensity of infection with *S. mansoni* from heavy to light, control as a public health issues has not been achieved, thus we recommend biannual MDA, in addition to integrated multi-sectoral efforts with increased access to safe water, sanitation and hygiene, health education and snail control.

1516

PERSISTENT HOTSPOTS OF *SCHISTOSOMA MANSONI* INFECTIONS AFTER 14 YEARS OF MASS DRUG ADMINISTRATION IN UGANDA: OPERATIONAL OR BIOLOGICAL FAILURES?

Jessica Clark[1], Christina L. Faust[2], Michelle Clements[3], Charlotte M. Gower[4], Moses Adriko[5], Moses Arinaitwe[6], Annet Enzaru[6], Annet Namukuta[6], Thomas Crellen[7], Adrina Nankasi[6], Candia Rowel[6], Aida Wamboko[6], Fred Besigye[6], Rachel Francoeur[1], Lauren V. Carruthers[1], Narcis B. Kabatereine[4], Alan Fenwick[4], Edridah M. Tukahebwa[6], Joanne P. Webster[8], Poppy H. L. Lamberton[1]

[1]*University of Glasgow, Glasgow, United Kingdom,* [2]*The Center for Infectious Disease Dynamics, Pennsylvania State University, State College, PA, United States,* [3]*Schistosomiasis Control Initiative, Imperial College, London, United Kingdom,* [4]*Imperial College, London, United Kingdom,* [5]*Vector Control Division, Minstry of Health, Kampala, Uganda,* [6]*Vector Control Division, Ministry of Health, Kampala, Uganda,* [7]*Big Data Institute, Oxford, United Kingdom,* [8]*University of London, Royal Veterinary College, London, United Kingdom*

Detrimental effects on planetary health disproportionately impact communities trapped in the cycle of poverty. Schistosomiasis is a disease inherently linked to this cycle, as transmission is a function of poor water quality, sanitation and hygiene (WASH) conditions. Praziquantel mass drug administration (40mg/kg dose) in school-age children and at-risk adults has been the mainstay of Ugandan schistosomiasis control for over a decade. Though treatment has been shown to be successful at reducing short-term prevalence and infection intensity, evidence from multiple high endemicity communities suggests that this relief is short lived, with prevalence and infection intensity returning to baseline values in as little as six months. To reach impending targets of schistosomiasis elimination it is imperative to understand what causes such rapid infection resurgence. We use a unique 14-year longitudinal dataset to determine whether these bounce back dynamics are symptomatic of a programmatic failure (e.g. treatment coverage) or a medical/biological failure (e.g. low drug efficacy). We used state-space modelling to quantify the temporal dynamics of the environmental force of infection. We then used a subset of the same data, to infer unobservable clearance and reinfection dynamics over the duration of MDA. We hypothesise that sustained, or increasing, environmental force of infection, and rapid reinfection is indicative of a programmatic failure where increased coverage and frequency of treatment could reduce overall community infection levels. Alternatively, we suggest that undulations in the force of infection over time accompanied by incomplete clearance is representative of a medical failure through incomplete clearance. In this scenario providing higher praziquantel doses (60mg/kg) or split doses over two consecutive days could improve drug efficacy. In both scenarios increasing the frequency of praziquantel treatment, providing community-wide coverage and improving WASH facilities would help communities reach WHO elimination targets.

1517

SCHISTOSOMIASIS: A DATA-DRIVEN ANALYSIS OF SYMPTOMS

Goylette Chami[1], Edridah Tukahebwa[2], Narcis Kabatereine[2]

[1]*University of Oxford, Oxford, United Kingdom,* [2]*Vector Control Division, Ministry of Health, Kampala, Uganda*

Prevention of morbidity rather than treatment is the focus of schistosomiasis control efforts. Consequently, morbidity often is not monitored in routine mass drug administration campaigns. More neglected is the landscape of cooccurring conditions with schistosomiasis. To develop integrated disease interventions, improve the identification of clinical signs for early diagnosis, and accurately estimate morbidity burden, there is a need to better understand the common clusters of conditions that cooccur in schistosomiasis endemic areas. A starting point for scoping cooccurring conditions is the analysis of clinical symptoms. A community-wide study of symptoms was conducted for individuals aged 1+ years in 17 rural villages of Mayuge District, Uganda. This area is hyperendemic for *Schistosoma mansoni.* The study communities had received approximately 10 annual rounds of mass drug administration with praziquantel. Reported symptoms were mapped to the Human Phenotype Ontology, and significantly cooccurring symptoms were analysed. Here we identify common clusters of symptoms. We show how clusters of symptoms vary based on schistosomiasis prevalence and the presence of hookworm coinfections. Well-known anatomical symptom clusters were identified. Symptom clusters also varied by social-ecological determinants of schistosome exposure. Importantly, evidence was found that suggested residual severe morbidities persist after repeated mass drug administration. The methods and findings presented here contribute information towards the design of integrated surveillance strategies, improved clinical diagnosis of schistosomiasis, and initial scoping strategies for cooccurring conditions.

1518

EFFECTS OF A WATER, SANITATION AND HYGIENE MOBILE HEALTH PROGRAM ON DIARRHEA AND CHILD GROWTH IN BANGLADESH: A CLUSTER-RANDOMIZED CONTROLLED TRIAL OF THE CHOBI7 MOBILE HEALTH PROGRAM

Christine Marie George[1], Shirajum Monira[2], Fatema Zohura[2], Elizabeth D. Thomas[1], M Tasdik Hasan[2], Tahmina Parvin[2], Khaled Hasan[1], Mahamud-ur Rashid[2], Nowshin Papri[2], Aminul Islam[2], Zillur Rahman[2], Raisa Rafique[2], Md. Sazzadul Islam Bhuyian[2], Ronald Saxton[1], Alain Labrique[1], Kelsey Alland[1], Indrajeet Barman[2], Fatema Tuz Jubyda[2], Marzia Sultana[2], Fatema-Tuz Johura[2], Lubaba Sharin[2], Lubaba Sharin[2], Md. Abul Hasem Khan[3], Sanya Tahmina[3], Farzana Munmun[3], David A. Sack[1], Jamie Perin[1], Munirul Alam[2]

[1]*Johns Hopkins University, Baltimore, MD, United States,* [2]*International Center for Diarrheal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh,* [3]*Bangladesh Ministry of Health and Family Welfare, Dhaka, Bangladesh*

The Cholera-Hospital-Based-Intervention-for-7-days (CHoBI7) mobile health (mHealth) program was a cluster-randomized controlled trial of diarrhea patient households conducted in Dhaka, Bangladesh. Patients were block-randomized to three arms: standard recommendation on oral rehydration solution use; health facility delivery of CHoBI7 plus mHealth (no home visits); and health facility delivery of CHoBI7 plus two home visits and mHealth. The primary outcome was reported diarrhea in the past two weeks collected monthly for 12 months. The secondary outcomes were stunting, underweight, and wasting at a 12 month follow-up. Analysis was intention-to-treat. The trial is registered at ClinicalTrials.gov (NCT04008134). Between December 4, 2016 and April 26, 2018, 2626 participants in 769 households were randomly allocated to three arms: 849 participants to standard message, 886 to mHealth with no home visits, and 891 to mHealth with two home visits. Children under five years had significantly lower 12-month diarrhea prevalence in both the mHealth with two home visits arm (Prevalence Ratio(PR): 0.73 (95% Confidence Interval(CI): 0.61, 0.87)) and the mHealth with no home visits arm (PR: 0.82 (95% CI: 0.69, 0.97)). Children under 2 years were significantly less likely to be stunted in both the mHealth with two home visits arm (33% vs. 45%, Odds Ratio(OR): 0.55, 95% CI: 0.31, 0.96) and the mHealth with no home visits arm (32% vs. 45%, OR: 0.55, 95% CI: 0.31, 0.96) compared to children in the standard message arm. The CHoBI7 mHealth program lowered pediatric diarrhea and stunting among diarrhea patient households over a 12 month period. Our findings suggest that mHealth can be used as a promising tool to improve child health.

1520

LEAD IS A POTENT NEUROTOXIN: DEVELOPING AN INTERVENTION TO REDUCE LEAD EXPOSURE AMONG PREGNANT AND LACTATING WOMEN IN BANGLADESH

Mahbubur Rahman[1], Tania Jahir[1], Helen Pitchik[2], Stephen P. Luby[3], Jesmin Sultana[1], AKM Shoab[1], Tarique Md Nurul Huda[1], Md Saiful Islam[1], Farzana Yeasmin[1], Musa Baker[1], Dalia Yeasmin[1], Syeda Nurunnahar[1], Peter J. Winch[4], Jenna Forsyth[3]

[1]*International Center for Diarrheal Diseases Research, Bangladesh (icddr,b), Dhaka, Bangladesh,* [2]*University of California Berkeley, Berkeley, CA, United States,* [3]*Stanford University,USA, Stanford, CA, United States,* [4]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States*

Lead exposure is harmful at any time in life, but pre and postnatal exposure is particularly detrimental to child cognitive development. In rural Bangladesh multiple household-level lead exposures may pose a risk to cognitive development. We developed and evaluated a theory based intervention to reduce household lead exposure among pregnant and mothers of children <2 years of age within a cluster randomized trial of an integrated intervention including child stimulation and nutrition. Our primary aim was to increase awareness and reduce lead exposure from food stored in lead soldered cans and turmeric adulterated with lead based coloring agent, and second aim was to increase intake of calcium and iron rich foods. We conducted formative research in Kishoreganj and Mymensingh districts, and developed theory-based behavioral recommendations to reduce exposure to these sources of lead. Community health workers delivered the intervention through group session and home visit. We piloted this intervention in 31 villages (N=621) from October 2017 to May 2018. To assess knowledge and self-reported behavior between control and intervention groups we administered a in-person household survey along with interviews and focus group discussions. Knowledge on lead at end line was 50% higher in the intervention group compared to the control group (p-value <0.001). Prevalence of safe turmeric consumption was 15% higher (46 vs. 31%, p=0.01) and safe food storage was 14% higher (95 vs. 81%, p=0.005) in the intervention arm. Prevalence of risky turmeric consumption was significantly lower in the intervention versus control arm (54 vs. 71%, p=0.009). Calcium and iron-rich food intake was also higher at the intervention arm. Qualitative findings revealed that the perceived benefit of reducing lead exposure was high in intervention arm. Participants reported that they were motivated to avoid long-term negative impacts that lead can have on their children's cognitive development. The study demonstrates how a group based, community health worker led, integrated intervention can effectively raise awareness about an unknown invisible toxin in rural Bangladesh.

1521

STRATEGIES TO CONNECT LOW-INCOME COMMUNITIES WITH THE PROPOSED SEWERAGE NETWORK OF THE DHAKA SANITATION IMPROVEMENT PROJECT, BANGLADESH

Mahbub Ul Alam[1], James B. Tidwell[2], Atik Ahsan[1], Ayesha Afrin[1], Sharika Ferdous[1], Fazle Sharior[1], Farhana Akand[1], Supta Sarker[1], Guy Norman[3], Mahbubur Rahman[1]

[1]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh,* [2]*Harvard Kennedy School of Government, Cambridge, MA, United States,* [3]*Water & Sanitation for the Urban Poor (WSUP), London, United Kingdom*

The Dhaka Sanitation Improvement Project (DSIP) supports the Dhaka Water Supply and Sewerage Authority (DWASA) in implementing the Sewerage Master Plan for Dhaka, supported by World Bank. This study assessed the feasibility of connecting low-income communities (LICs) with the proposed sewerage network under DSIP. We conducted 5 key informant interviews with DWASA and Dhaka City Corporation personnel, and 23 focus group discussions with landlords, tenants and CBOs from 18 LICs situated in the catchment area of proposed sewerage network. FGD participants' monthly income ranged from 118 to 177 USD, and the monthly house rent was 30-35 USD. Most respondents shared toilets among 10 or more households. Landlords were usually responsible for constructing toilets and for repair and maintenance. Toilets commonly discharged waste into an open water body nearby. The surrounding environment was polluted, and food and water were commonly contaminated by this wastewater, causing waterborne diseases. Prevention of overflow of faecal matter and wastewater leading to diseases were the perceived benefits of sewerage connection. The most common barriers for being connected were collective action failure due to disagreements about who should take initiative, settlement informality, and lack of space inside LICs. To sewer LICs will require improved toilet superstructures and main roads outside the LICs, along with construction of large communal septic tanks linked to the sewerage network where connection inside LICs is difficult. To support poor residents, income-based or area-based subsidies were recommended and monthly fees could be collected by dividing the bill equally within sharing households, or based on number of users per household. Participants recommended that government must work with other development partners/NGOs to ensure sewerage network connections and operation and maintenance. Awareness raising, although rarely done by the DWASA in the past, will be necessary through social media, short films, mosque-based dissemination or via open meetings in the community to increase demand for sewer connections among LIC residents.

1522

PATTERNS AND DRIVERS OF SUSTAINED AND GAINED HOUSEHOLD SANITATION ACCESS BETWEEN 2015 AND 2017 AMONG HOUSEHOLDS IN THE TUMIKIA TRIAL IN KWALE COUNTY, KENYA

Hugo Legge[1], Stella Kepha[1], Stefan S. Witek-McManus[1], Katherine E. Halliday[1], Carlos Mcharo[2], Hajara El-Busaidy[3], Redempta Muendo[3], Th'uva Safari[2], Charles S. Mwandawiro[2], Sultani H. Matendechero[4], Rachel L. Pullan[1], William E. Oswald[1]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*Eastern and Southern Africa Centre of International Parasite Control, Kenya Medical Research Institute, Nairobi, Kenya,* [3]*Ministry of Health, County Government of Kwale, Kwale, Kenya,* [4]*Division of Vector Borne and Neglected Tropical Diseases Unit, Ministry of Health, Nairobi, Kenya*

Many sanitation interventions suffer from poor sustainability of outcomes. Failure to rehabilitate or replace non-functioning facilities risks re-exposing communities to harmful environmental pathogens and inhibits progress towards SDG6. Relatively little is known about what factors drive sustained access beyond project lifespans, and few studies have examined household sanitation access longitudinally outside of intervention settings. Using data from a cohort of 1405 households enrolled in the TUMIKIA project, a cluster-randomized trial in Kenya evaluating the effectiveness of school-based deworming versus community-wide treatment with no active WASH component, we investigated factors associated with change in sanitation access between 2015 and 2017. Sanitation access was defined as: access to an improved or unimproved facility within the compound that was functional and in-use. A range of contextual, psychosocial, and technological covariates were included in logistic regression models with random intercepts to estimate associations between covariates and; 1) likelihood of sustaining sanitation access; and 2) likelihood of gaining sanitation access. Across the two years, 28.3% households sustained sanitation access, 4.7% lost access, 17.7% gained access and 49.2% maintained no access. Factors associated with an increased likelihood to sustain sanitation access included: household access to an unshared facility; access to a facility with a solid, washable slab; and being located in a rural area. Factors associated with an increased likelihood to gain sanitation access included: households with higher socioeconomic status; households whose head had at least secondary school education; and households that were located in clusters with higher community-wide coverage of sanitation access in 2015. Our results show that in this setting, among other factors, private access and durable sanitation platforms should be prioritized in order to promote sustainability, and they support findings from previous studies highlighting the importance of community-wide norms in determining initial adoption of sanitation.

1523

ENDLINE EVALUATION OF A WASH IN SCHOOL PROGRAM FOR ABSENTEEISM, DIARRHEA AND RESPIRATORY INFECTIONS AMONG STUDENTS IN SECONDARY SCHOOLS IN BANGLADESH

Debashish Biswas[1], Abul K. Shoab[1], Mahbub-Ul Alam[1], Mahbubur Rahman[1], Mirza M. Sultana[2], Mahfuj-ur Rahman[2], Aftab Opel[2], Leanne Unicomb[1]

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

In 2020, we evaluated a WASH in School program, implemented between 2017 and 2019 by WaterAid Bangladesh among 300 schools in Rangpur, Gaibandha and Satkhira districts in Bangladesh. The evaluation aimed to estimate potential health and school attendance impact to inform intervention scale up. No baseline data were collected so we enrolled students from intervention schools and propensity score matched non intervention (control) schools where matching scores were derived using school enrollment, geographical locations, and infrastructure of the schools. From randomly selected intervention schools from 3 geographic locations (n=26) and 26 matched controls a total of 1,560 students were enrolled between January and March 2020. Data were collected during student interview to assess recent absenteeism (last 30 days), respiratory symptoms in last 7 days and any history of diarrhoea in last 14 days from the date of interview. Odds ratios (OR) and 95% confidence intervals were determined using generalized linear model, controlling for cluster level effects and adjusting for individual and school level factors. Significantly fewer students missed at least one school-day in last 30 days in intervention schools (68%) compared to students from control schools (75%) (OR=0.72, 95%CI=0.59, 0.88). The prevalence of student diarrhea (defined as three or more loose or watery stools in a day) in the previous two weeks was lower in intervention than in the control schools (6% vs 9%, OR=0.82, 95%CI=0.68, 0.99). Similarly, students from intervention schools had fewer respiratory infections (defined as fever with cough/runny nose/difficult breathing) than control schools (28% vs 34%, OR=0.71, 95% CI=0.70, 0.72). Using a control-matched post intervention study design, courtesy bias among intervention school students and selection bias when recruiting control schools cannot be ruled out. Nevertheless, intervention school students reported fewer illnesses and school absence suggesting that the intervention had health impact and is worth exploring for scale up. Implementers should assume that health impact may be more modest in a scaled intervention.

1524

HAND HYGIENE DURING CHILDBIRTH: A MIXED-METHODS OBSERVATIONAL STUDY IN CAMBODIA

Robert Dreibelbis[1], Yolisa Nalule[1], Ponnary Pons[2], Helen Buxton[1], Channa Sam Ol[2], Alison Macintyre[3], Leang Supheap[4], Ir Por[4]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*WaterAid Cambodia, Phnom Penh, Cambodia,* [3]*WaterAid Australia, Melbourne, Australia,* [4]*National Institutes of Public Health, Cambodia, Phnom Penh, Cambodia*

Infection acquired during childbirth remain a leading cause of maternal and neonatal mortality. Proper hand hygiene is critical to reducing these infections and is a central component of all infection prevention and control (IPC) strategies. However, there is limited robust data on hand hygiene compliance during the process of childbirth and limited exploration of hand hygiene determinants. We completed structured, direct observations of midwife hand hygiene practices during childbirth for 45 mothers with normal deliveries in Kampong Chhnang Province, Cambodia. Observed hand hygiene practices were compared against WHO and Cambodian guidelines. Participatory interviews explored the determinants of observed hand hygiene practices and identify potential intervention strategies. Hand hygiene infrastructure and IPC supplies were available and accessible during all observed deliveries; however, adherence to hand hygiene guidelines was low. Of 95 observed vaginal examinations, only 23% were completed following full hygiene protocol (hands washed with soap and clean gloves worn) and 25% were completed when aseptic technique had been invalidated (no hand hygiene action taken and/or hands and gloves recontaminated prior to the procedure). Similar patterns were observed for hand hygiene at the initiation of delivery flows (n = 105). Recontamination of gloves during birth was common. For the 21 delivery flows that were initiated following proper hand hygiene protocol, midwives were able to maintain proper hand hygiene throughout childbirth in only 8 (38%). Qualitative results show that midwives understand proper hygiene protocol and its importance. However, midwives report time pressures during labor and delivery as a factor contributing to sub-optimal hygiene practices, such as "double gloving" as a shortcut for hand hygiene. Midwives also rely on sensory cues (ie: visible contamination) as a trigger for hand hygiene. Interventions that make hand hygiene easier and more convenient for midwives and incorporate environmental cues or "nudges" - should be included as part of larger programs to strengthen IPC during childbirth.

1525

DYSREGULATION OF ANGIOPOIETIN-TIE-2 AXIS IN UGANDAN CHILDREN HOSPITALIZED WITH PNEUMONIA

Ran Zhang[1], Urvi Rai[1], Nafeesah Bte Mohamed Ibrahim[1], Yanni Amazouz[1], Jeremy Soo[1], Andrea L. Conroy[2], Sophie Namasopo[3], Robert O. Opoka[4], Ravi Barghava[1], **Michael T. Hawkes**[1]

[1]*University of Alberta, Edmonton, AB, Canada,* [2]*Indiana University, Indianapolis, IN, United States,* [3]*Jinja Regional Referral Hospital, Kampala, Uganda,* [4]*Makerere University, Kampala, Uganda*

Pneumonia is the leading cause of death in children under 5, with the highest burden in resource-limited countries. Endothelial activation occurs in pneumonia and can be assessed using quantitative levels of biomarkers angiopoietin (Ang)-1 and Ang-2. We examined admission levels of Ang-1 and Ang-2 in pediatric pneumonia and their association with disease severity and outcome. This was a prospective cohort study of children with hypoxemic pneumonia admitted to two hospitals in Uganda. Clinical, radiographic, and microbiologic characteristics were measured at admission. Disease severity was assessed using the Respiratory Index of Severity in Children (RISC). Plasma levels of Ang-1 and Ang-2 were quantified by enzyme-linked immunosorbent assay. Vital signs, oxygen supplementation, and mortality were assessed prospectively. We included 65 patients (43% female) with median age 19 months (IQR 8-24). Admission Ang-2/Ang-1 ratio directly correlated with RISC (ρ=0.32, p=0.008) and lactate level (ρ = 0.48, p<0.001). Ang-2/Ang-1 ratio was higher in pneumococcal pneumonia than viral RTI (0.19 [IQR: 0.076-0.54] vs. 0.078 [IQR: 0.027-0.11]; p=0.03). Elevated Ang-2/Ang-1 ratio (>0.084) was associated with prolonged tachypnea (HR 0.50 (95%CI 0.29- 0.87), p=0.02), fever (HR 0.56 (95%CI 0.33 to 0.96), p=0.02), longer duration of oxygen therapy (HR 0.59 (95%CI 0.35-0.99), p=0.04), and hospital stay (HR 0.43 (95%CI 0.25-0.74), p=0.001). The Ang-2/Ang-1 ratio at admission was higher in fatal cases relative to survivors (0.36 [IQR: 0.17-0.58] vs. 0.077 [IQR: 0.025-0.19]; p= 0.05) In conclusion, endothelial activation in hypoxemic pediatric pneumonia, reflected by high plasma Ang-2/Ang-1 ratio, is associated with disease severity, prolonged recovery time, and mortality.

1527

RESPIRATORY VIRUS INFECTIONS FROM THE SENTINEL ENHANCED DENGUE SURVEILLANCE SYSTEM, 2016-2019

Rachel M. Rodríguez-Santiago[1], Robert Rodríguez-González[1], Kelmie Torres-Rivera[1], Vanessa Rivera-Amill[1], Luisa I. Alvarado-Domenech[1], **Laura Adams**[2]

[1]*Ponce Health Sciences University, Ponce, PR, United States,* [2]*Dengue Branch, Centers for Disease Control and Prevention Division of Vector-Borne Diseases, San Juan, PR, United States*

Respiratory tract infections have an important impact on public health, causing an estimated ~500 million infections per year in the United States. Respiratory viruses tend to have antigenic variations leading to outbreaks, epidemics or pandemics in naïve populations. The Sentinel Enhanced Dengue Surveillance System (SEDSS) is a research program for the study of the epidemiology, clinical manifestations, diagnosis and outcomes of dengue and other acute febrile illnesses (AFI). SEDSS is located in a tertiary care hospital and affiliated urgent clinic in the southern region of Puerto Rico with a population base of ~ 500,000. After informed consent, participants provide information on the course of their illness, and blood, urine, and naso/oro-pharyngeal specimens for RT-PCR testing of the following respiratory viruses: influenza A/B, parainfluenza 1/3, adenovirus, human respiratory syncytial virus (HRSV) and human meta-pneumo virus. This study aims to describe and compare the distribution of respiratory viruses by age, sex, co-infections and disposition in the most recent 4 year period. During 2016-2019, 20,270 participants were enrolled in SEDSS; 52% were females. Among all participants, 29% (n=5,947) had at least one respiratory virus identified by RT-PCR. Of this group, respiratory virus co-infections were identified among 163 (3%) participants. Children 0-4 years of age represent 33% of SEDSS participants; of this group, 37% had a confirmed respiratory infection. The most frequently identified respiratory viruses were influenza A (33.5%), adenovirus (15.4%), influenza B (14.7%), and HRSV (14.6%). As a future direction, SARS-CoV 2 testing during the COVID-19 pandemic will be implemented. Epidemiological patterns and clinical features of respiratory viruses from previous years will be compared with the patterns presented by COVID-19. This information can be useful to clinicians and public health officials in understanding COVID-19 in the context of other common respiratory virus infections.

1528

PREVALENCE, RISK FACTORS AND OUTCOME OF HOSPITAL ACQUIRED PNEUMONIA IN YOUNG BANGLADESHI CHILDREN

Mohammod J. Chisti, Abu S. Shahid, K. M. Shahunja

International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh

Hospital acquired pneumonia (HAP) is common specifically in developing countries and associated with high mortality. Data on HAP in children under five hospitalized for diarrhea are scarce. We sought to evaluate the prevalence, risk factors and outcome of HAP in these children. We compared demographic, clinical, laboratory, and blood culture data in a cohort of young children (< 5 years) admitted to Dhaka Hospital of the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) from August 2013 to December 2017 between the children with (cases) and without HAP (controls) diagnosed both clinically and radiographically. HAP was defined if a child developed new episode of pneumonia after at least 48 hours of hospitalization. Two fold controls were selected randomly. Among a total of 4101 study children, 281(6.9%) were cases and 562 were controls. The mortality rate was significantly higher among the cases than the controls (85 vs. 4%, p=0.014). In multivariate logistic regression analysis, persistent diarrhea (odds ratio [OR] 2.83, 95% CI 1.34-5.99; p=0.007), severe acute malnutrition (OR 2.04, 95% CI 1.35-3.08; p=0.001), bacteremia (OR 2.24, 95% CI 1.27-3.96; p=0.005), and hospitalization >5 days (OR 2.04, 95% CI 2.83-7.71; p<0.001) were identified as risk factors for HAP. Thus, HAP was 7% in children under five hospitalized for diarrhea and was associated with a higher mortality rate compared to children with other diagnoses. Early identification of simple risk factors for HAP and their prompt treatment may help to reduce HAP related fatal consequences.

1529

PERFORMANCE EVALUATION OF THE XPERT MTB/RIF ULTRA ASSAY ON POSTMORTEM NASOPHARYNGEAL SPECIMENS TO IDENTIFY SUSPECTED AND UNSUSPECTED TUBERCULOSIS DEATHS AMONG HOSPITALIZED PATIENTS AT AUTOPSY IN NORTHERN TANZANIA

Cristina R. Costales[1], John A. Crump[2], Alex R. Mremi[3], Patrick T. Amsi[3], Manuela Carugati[4], Deng Madut[4], Ann M. Nelson[5], Venance P. Maro[3], Matt P. Rubach[4]

[1]*Keck School of Medicine of the University of Southern California, Los Angeles, CA, United States,* [2]*Centre for International Health, University of Otago, Dunedin, New Zealand,* [3]*Kilimanjaro Christian Medical Centre, Moshi, United Republic of Tanzania,* [4]*Division of Infectious Diseases and International Health, Duke University, Durham, NC, United States,* [5]*Inpala Consulting, Washington, DC, United States*

The diagnosis of tuberculosis (TB) in severely ill patients and decedents in low-resource, high tuberculosis incidence countries is compromised by the difficulty in obtaining adequate lower respiratory tract samples. With autopsy-confirmed TB as our standard, we sought to assess the diagnostic performance of the Xpert MTB/RIF Ultra assay using upper respiratory tract nasopharyngeal (NP) specimens collected postmortem. From October 2016 through May 2019, we conducted a prospective postmortem study, by complete diagnostic autopsy (CDA) or minimally invasive tissue sampling (MITS) procedures, at two referral hospitals

in northern Tanzania. We collected NP specimens by swabbing the posterior nasal turbinate through the nostril prior to autopsy, then tested by Ultra assay. At autopsy we collected lung, liver, and, when possible, cerebrospinal fluid for mycobacterial culture and histopathlogy. Isolation of *M. tuberculosis* complex (MTBc) was considered definite evidence of TB, whereas histopathology findings, including necrotizing granulomatous inflammation or positive acid-fast bacilli tissue staining, were considered probable evidence of TB. We reviewed clinically-determined death certificates. Of 205 decedents included in the analysis, 78 (38.0%) were female and median (range) age at death was 45 (<1, 96) years. Twenty-seven (13.2%) of the 205 decedents were diagnosed with TB at autopsy, including 22 (81.5%) definite and 5 (18.5%) probable. Twenty-three (85.2%) of these 27 had disseminated disease. Ultra assay detected MTBc from NP swabs in 21 (77.8%) of 27 decedents with definite or probable TB for a sensitivity of 70.4% and a specificity of 98.9%. The sensitivity was 81.8% when restricted to those with definite TB. Fourteen (66.7%) of 21 MTBc Ultra detections occurred in decedents without TB as a death certificate diagnosis. Ultra testing on postmortem NP specimens was highly specific for identifying in-hospital TB deaths, and accurately detected clinically unsuspected TB deaths. This approach has the potential to improve TB death enumeration in high burden countries already using the Ultra assay in their TB control programs.

1530

THE POISONED CHALICE: A BIOMIMETIC 'TROJAN HORSE' PLATFORM FOR PRECISION KILLING OF MDR TUBERCULOSIS

Andrew W. Simonson[1], John N. Alumasa[1], Agustey S. Mongia[1], Matthew R. Aronson[1], Michael D. Howe[2], Bailey Klein[1], Sarah Almarzooqi[1], Adam Bolotsky[1], Aida Ebrahimi[1], Anthony D. Baughn[2], Kenneth C. Keiler[1], Scott H. Medina[1]

[1]*Pennsylvania State University, University Park, PA, United States,* [2]*University of Minnesota, Minneapolis, MN, United States*

Multi-drug resistant tuberculosis (MDR-TB) is a growing threat, with the global burden worsening by 20% per year due to epidemics driven by direct transmission. Thus, development of novel therapies to rapidly clear pulmonary TB and prevent transmission is a global health priority. We have developed a TB-specific host defense peptide that acts by mimicking mycobacterial transmembrane porins to selectively disrupt the pathogen's cell envelope. Using MspA as a porin template, we rationally designed the *de novo* peptide MAD1 and demonstrate its ability to rapidly and selectively kill TB pathogens through physical mechanisms not shared by conventional therapies. As a result of its membrane-active mechanisms, MAD1 synergistically enhances the potency of poorly permeable TB antibiotics, particularly the second-line agent Moxifloxacin (MOX). However, despite the therapeutic potential of MAD1, systemic delivery of membrane-active peptides remains challenged by off-target distribution and proteolytic instability. Thus, we developed an extracellular matrix (ECM)-inspired nano-aerosol, or 'aerogel', designed to preferentially deliver combinatorial peptide therapies to mycobacteria and infected host cells to rapidly clear pulmonary MDR-TB. This is achieved by electrostatically assembling MAD1 with the ECM carbohydrate hyaluronic acid (HA). HA-MAD1 assembly imparts aerogels with several complementary bioactive functions: (1) Respiratory pathogens use HA as a carbon source during virulence, thereby enabling therapeutic aerogels to rapidly engage invading TB pathogens and operate as a molecular 'Trojan Horse'. (2) HA avidly binds CD44 receptors on the surface of macrophages, triggering endocytic uptake of the particles to sterilize persister pathogens from infected host immune cells. (3) Electrostatic assembly of MAD1 and HA allows facile encapsulation of combinatorial drug cocktails for synergistic therapy. Together, these unique features define aerogels as a novel, aerosol drug delivery platform with broad potential for rapid, potent and precise clearance of MDR-TB pulmonary disease, with negligible host toxicity.

1531

CASCADE ANALYSIS OF HOUSEHOLD CONTACT INVESTIGATION FOR TUBERCULOSIS IN CALI, COLOMBIA

Gustavo Diaz[1], Angela M. Victoria[2], Amanda J. Meyer[3], Yessenia Niño[4], Lucy Luna[4], Beatriz E. Ferro[2], J. Lucian Davis[5]

[1]*Centro Internacional de Entrenamiento e Investigaciones Medicas-CIDEIM & Alianza TB, Cali, Colombia,* [2]*Departamento de Salud Pública y Medicina Comunitaria, Universidad Icesi & Alianza TB, Cali, Colombia,* [3]*Department of Epidemiology of Microbial Diseases, Yale School of Public Health, New Haven, CT, United States,* [4]*Secretaría de Salud Pública Municipal de Santiago de Cali-Programa de Control de Micobacterias & Alianza TB, Cali, Colombia,* [5]*Department of Epidemiology of Microbial Diseases, Yale School of Public Health & Pulmonary, Critical Care and Sleep Medicine Section, Yale School of Medicine, New Haven, CT, United States*

Tuberculosis (TB) contact investigation is recommended to increase TB diagnosis and facilitate the delivery of isoniazid preventive therapy (IPT), but the quality of its delivery in routine practice is unknown. We aimed to evaluate the efficiency and effectiveness of TB contact investigation in Cali, Colombia, an intermediate TB-burden country seeking to eliminate TB. We performed a cross-sectional study of all index TB patients diagnosed in 2017 who had ≥1 household contact (HHC). We evaluated several key process indicators, including the proportions of 1) index TB patients receiving a household visit; 2) HHC referred for TB evaluation who visited clinics; 3) HHC newly diagnosed with active TB; and 4) HHC initiating IPT. We estimated the cumulative probability of a household contact with symptomatic TB being identified and tested. We also ran multivariable analyses to identify individual predictors of completion of 1) a household visit; and 2) a referral visit to the clinic. Among registered TB patients, 68% (759/1120) were eligible for HHC investigation. 582 (77%) received a household visit. Adjusting for household clustering, multivariable analysis found that odds of household visit completion was significantly lower in male patient (adjusted odds ratio (aOR) 0.6, 95% CI 0.4-0.9, p=0.02) and patients living in the Western zone (aOR 0.5, 95% CI 0.3-0.8, p<0.01). Among screened HHC, 31% (582/1880) met ≥1 criteria for clinic referral for TB evaluation, but only 47% (271/582) completed clinic visits. The cumulative probability of an HHC with active TB completing testing for TB was only 36%. Odds of referral completion were higher among contacts with productive cough (aOR 21.6, CI 7.1-66, p<0.01) and those from Cali's Western zone (aOR 4.1, 95% CI 1.2-15, p=0.03). Only 5/1880 (0.3%) were diagnosed with active TB, while 16% (17/103) children <5 years and none (0/8) of the contacts living with HIV started IPT. Rigorous analysis of administrative data identified substantial gaps in the efficiency and effectiveness of routine TB contact investigation in Cali, pointing out multiple areas for quality improvement.

1532

A PHASE I OPEN-LABEL, DOSE-ESCALATION CLINICAL TRIAL TO EVALUATE THE SAFETY, TOLERABILITY AND IMMUNOGENICITY OF THE MARBURG CHIMPANZEE ADENOVIRUS VECTOR VACCINE, VRC-MARADC087-00-VP (CAD3-MARBURG), IN HEALTHY ADULTS

Melinda J. Hamer[1], Julie A. Ake[1], Martin R. Gaudinski[2], LaSonji A. Holman[2], Christine E. Lee[1], Alicia Widge[2], Anne C. Preston[1], Josephine H. Cox[2], Jack N. Hutter[1], James E. Moon[1], Steven A. Schech[3], Victoria Kioko[3], Casey Storme[3], Paul T. Scott[1], Kayvon Modjarrad[1], Merlin Robb[3], Nancy J. Sullivan[2], Julie E. Ledgerwood[2]

[1]*Walter Reed Army Institute of Research, Silver Spring, MD, United States,* [2]*Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States,* [3]*Walter Reed Army Institute of Research; Henry M. Jackson Foundation for the Advancement of Military Medicine, Silver Spring, MD, United States*

In 2018, the World Health Organization identified Marburg virus as an important emerging infectious disease requiring urgent research and development. Here we report results from a first-in-human trial evaluating a replication-deficient recombinant chimpanzee adenovirus

type-3 vectored Marburg virus vaccine (cAd3-Marburg) in healthy adults. This was a phase 1, open-label, dose-escalation human clinical trial of cAd3-Marburg with an encoded wild-type Angola glycoprotein insert. 40 healthy adults aged 18 -50 (inclusive) were recruited and enrolled into two 20-subject groups, each receiving a single intramuscular dose of either 1×10^{10} or 1×10^{11} particle units. Primary safety endpoints were evaluated through 48 weeks following vaccination. Immunogenicity endpoints included assessment of antibody (via ELISA) and T cell responses, with the principal time point at four weeks after vaccination. All subjects completed vaccination, with 40 (100%) completing principal immunogenicity time point, and 37 (92.5%) completing full follow-up through Day 336. There were no significant safety concerns identified. In the 1×10^{11} particle unit dose group, 3 (15%) subjects had transient fever following vaccination, 2 (10%) subjects developed transient, asymptomatic mild leukopenia, and 1 (5%) subject developed moderate transient neutropenia. No similar abnormalities were noted in the 1×10^{10} particle unit dose group. Glycoprotein-specific antibodies were induced in the majority of subjects in both groups (34/40 total trial subjects) at the prospectively defined week four immunogenicity endpoint with a trend toward greater magnitude in the 1×10^{11} particle unit dose group. This novel cAd3-Marburg vaccine was safe and immunogenic in this trial. The safety assessment was reassuring and similar to previous cAd3-vectored filovirus vaccines. Nearly all subjects produced a glycoprotein-specific antibody response after a single vaccination with cAd3-Marburg. Further evaluation and optimization of this vaccine candidate is warranted.

1533

EVALUATING THE LONG-TERM IMMUNOGENICITY OF ADENOVIRAL AND MVA VECTORED EBOLA VACCINE SCHEDULES AND RESPONSE TO A BOOSTER DOSE OF AD26.ZEBOV

Catherine Clare Smith[1], Rebecca Makinson[2], Mehreen S. Datoo[2], Souleymane Mboup[3], Birahim P. Ndiaye[3], Amy Flaxman[4], Tandakha Dieye[3], Josephine Badiane[3], Juliana Ballaminut[1], Jamie Burbage[5], Katja Pfafferot[1], Sarah Kelly[1], Duncan Bellamy[6], Charlotte Black[1], Rachael Drake-Brockman[1], Parvinder Aley[1], Katrina Pollock[7], David Lewis[7], Adrian V. Hill[4], Katie Ewer[6], Matthew D. Snape[5]

[1]*Oxford Vaccine Group and NIHR Oxford Biomedical Research Centre, University of Oxford, Oxford, United Kingdom,* [2]*Jenner Institute, University of Oxford, Oxford, United Kingdom,* [3]*Institut de Recherche en Santé, de Surveillance Epidemiologique et de Formation (IRESSEF)., Dakar, Senegal,* [4]*Jenner Institute, University of Oxford, Oxford, United Kingdom,* [5]*Oxford Vaccine Group and NIHR Oxford Biomedical Research Centre , University of Oxford, Oxford, United Kingdom,* [6]*Jenner institute, University of Oxford, Oxford, United Kingdom,* [7]*NIHR/Wellcome Trust Imperial Clinical Research Facility Hammersmith Hospital, London, United Kingdom*

Regimens based on viral-vectored Ebola vaccines including rVSV.ZEBOV and Ad26.ZEBOV/MVA-BN-Filo, have been recommended[OMV[1] approved by SAGE for use in the current outbreak in the Democratic Republic of Congo. Data on the longevity of immune responses to viral-vectored prime- boost Ebola vaccine regimens, including a booster dose of Ad26.ZEBOV, are vital to inform the future use of vaccine combinations in at-risk areas to help curtail further epidemics and ensure healthcare workers are protected. We undertook open-label, follow-on studies in the U.K. and Senegal assessing the durability of the immune response after prime-boost regimens of Adenoviral and MVA-vectored Ebola vaccines, and the effect of a booster dose of Ad26.ZEBOV. In the UK 47 healthy volunteers previously immunised with a Chimpanzee Adenovirus 3 vectored vaccine (ChAd3-EBO-Z) in combination with either MVA-BN-Filo (N=25), an alternative MVA vectored vaccine (MVA-EBO-Z) (N=19) or Ad26.ZEBOV (N=3) were seen 42-62 months after primary immunisation, 29 of whom received a booster dose of Ad26.ZEBOV at this time. In Senegal, 28 participants previously immunised with ChAd3-Ebo-Z and MVA-EBO-Z all received an Ad26.ZEBOV booster dose 48 months after primary immunisation. At 42-62 months after primary immunisation, 32 of 47 UK participants (68%) retained detectable Ebola glycoprotein-specific T cells (measured by IFN-γ ELISPOT), and 18 of 47 (38%) were seropositive (IgG measured by ELISA). Amongst participants receiving an Ad26.ZEBOV boost the median T cell response rose from 99 SFC/million at baseline to 2138 at day 7 and 688 at day 28. Geometric mean IgG concentrations rose from 122 EU to 13599 (day 7) and 14927 (day 28), responses indicative of long-lived humoral memory induced by prior immunisation with replication-deficient vectored vaccines. Additional immunogenicity data from the Senegalese participants will be presented. These data demonstrate that schedules containing viral vectored vaccines are able to induce highly sustained immune responses, which may be of relevance to other adenoviral-vectored vaccines currently in development, including those against RSV and SARS-CoV-2. [OMV[1]"approved" refers to approval by regulatory authorities

1534

MERCK RVSVÄG-ZEBOV-GP EBOLA VACCINE: UPDATED SAFETY, IMMUNOGENICITY, AND EFFICACY AND ESTIMATION OF THE CORRELATES OF PROTECTION

Jakub Simon[1], Stepen Kennedy[2], Barbara Mahon[3], Sheri Dubey[1], Rebecca Grant-Klein[1], Ken Liu[1], Jonathan Hartzel[1], Beth-Ann Coller[1], Carolee Welebob[1], Mary Hanson[1], Rebecca Grais[4]

[1]*Merck & Co., Inc., Kenilworth, NJ, United States,* [2]*Liberian Ministry of Health, Monrovia, Liberia,* [3]*Bill & Melinda Gates Foundation, Seattle, WA, United States,* [4]*Epicenter, Paris, France*

The recent Ebola virus disease (EVD) outbreak in the Democratic Republic of the Congo highlights the sustained threat of EVD morbidity and mortality where healthcare and vaccine delivery are challenging. The vaccine developed by Merck & Co., Inc., Kenilworth, NJ, USA jointly with multiple partners to prevent EVD is a live recombinant vesicular stomatitis virus (VSV) containing the *Zaire ebolavirus* glycoprotein (GP) in place of the VSV GP (rVSVΔG-ZEBOV-GP; ERVEBO™). Its safety, efficacy, or immunogenicity has been evaluated in 16 clinical trials, and the vaccine has been approved for use in humans by the European Medicines Agency, United States Food and Drug Administration, several African countries, and has been prequalified by the World Health Organization. More than 300,000 people have received the vaccine as of 11 March 2020 (time of abstract preparation). Updated immunogenicity data from three Phase 2/3 clinical trials conducted in Guinea, Sierra Leone, and Liberia during the 2013-2016 West African outbreak will be discussed with an emphasis on work aimed at identifying immune correlates of protection. Challenges relevant to relating data from non-human primates to humans as well as establishing correlates of protection for highly efficacious vaccines will be discussed.

1535

CHARACTERISTICS OF EBOLA VIRUS DISEASE SURVIVOR BLOOD AND SEMEN IN LIBERIA: SEROLOGY AND RT-PCR

Aaron Kofman[1], Susanne Linderman[2], Kaihong Su[3], Lawrence J. Purpura[1], Elizabeth Ervin[4], Shelley Brown[4], Maria Morales-Betoulle[4], James Graziano[4], Deborah L. Cannon[4], John D. Klena[4], Rodel Desamu-Thorpe[5], John Fankhauser[6], Romeo Orone[7], Moses Soka[8], Uriah Glaybo[8], Moses Massaquoi[9], Tolbert Nysenswah[9], Stuart T. Nichol[4], Jomah Kollie[9], Armah Kiawu[9], Edna Freeman[9], Giovanni Giah[9], Henry Tony[9], Mylene Faikai[9], Mary Jawara[9], Kuku Kamara[9], Samuel Kamara[8], Benjamin Flowers[8], Kromah L. Mohammed[9], David Chiriboga[10], Desmond E. Williams[11], Steven H. Hinrichs[3], Rafi Ahmed[2], Benjamin Vonhm[9], Pierre E. Rollin[4], Mary J. Choi[4]

[1]*Epidemic Intelligence Service, Viral Special Pathogens Branch, Division of High-Consequence Pathogens and Pathology, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Emory Vaccine Center, Emory University, Atlanta, GA, United States,* [3]*Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE, United States,* [4]*Viral Special Pathogens Branch, Division of High-Consequence Pathogens and Pathology, National Center for Emerging and Zoonotic*

Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, United States, [5]Office of Public Health Preparedness and Response, Center for Preparedness and Response, Centers for Disease Control and Prevention, Atlanta, GA, United States, [6]Santa Clara Valley Medical Center, San Jose, CA, United States, [7]ELWA Hospital, Samaritan's Purse, Monrovia, Liberia, [8]Ministry of Health, Liberia, Monrovia, Liberia, [9]Men's Health Screening Program, Monrovia, Liberia, [10]University of Massachusetts Medical School, Worcester, MA, United States, [11]Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, United States

Ebola virus (EBOV) is known to persist in the semen of male survivors of Ebola Virus Disease (EVD). However, the risk factors for prolonged EBOV persistence in semen are unknown. We conducted a study of male survivors of the 2014-2015 EVD outbreak in Liberia to understand host determinants for the long-term persistence of EBOV in semen, for which analysis is still underway. We report here a separate analysis of molecular and immunological characteristics for a subset of participants. Of 126 study participants for whom samples were available, all 126 participants tested negative for the presence of EBOV RNA in blood by qRT-PCR. The blood of 26 (22%) participants tested negative for the presence of EBOV-specific IgG antibodies by ELISA. EVD survivorship could be corroborated for 7 of the 26 EBOV IgG-negative participants, either through inclusion in the Liberian Ministry of Health's registry of laboratory-confirmed EVD survivors, detection of EBOV RNA in semen on at least one occasion, or both. Peripheral blood mononuclear cells (PBMCs) were collected from 23 of the 26 EBOV IgG-negative participants. Of these, 1 of the 23 participants had PBMCs which produced anti-EBOV-specific IgG antibodies upon stimulation with EBOV-specific GP and NP antigens. We conclude that the blood of EVD survivors, who do not meet the case definition for acute EVD, does not pose a risk for EBOV transmission. We also identified possible seroreversion in 7 laboratory-confirmed EVD survivors several years after resolution of acute infection, and evidence of persistent memory in the PBMCs of one EVD survivor. This suggests that immunogenicity to EBOV may exist along a spectrum in survivors of EVD, and absence of antibody response should not be automatically exclusionary in determining an individual's status as a survivor of EVD if other epidemiological and clinical data is consistent with a history of EVD.

1536

RHABDOMYOLYSIS, KIDNEY INJURY, AND MORTALITY IN EBOLA VIRUS DISEASE IN EASTERN DEMOCRATIC REPUBLIC OF THE CONGO

Claude Masumbuko Kasereka[1], Zubia Mumtaz[1], Andrea Conroy[2], Malengera Kambale[3], Tsongo Kibendelwa[4], Didier Mwesha[5], Michael T. Hawkes[1]

[1]*University of Alberta, Edmonton, AB, Canada, [2]Indiana University School of Medicine, Indianapolis, IN, United States, [3]Universite Catholique du Graben, Butembo, Democratic Republic of the Congo, [4]Universite de Kisangani, Kisangani, Democratic Republic of the Congo, [5]World Health Organization, Butembo, Democratic Republic of the Congo*

Skeletal muscle injury in Ebola virus disease (EVD) is scantily described but may play an important role in its pathogenesis. Rhabdomyolysis is reported in EVD patients, but its impact on morbidity and mortality remains unclear. We conducted a retrospective observational study of consecutive patients admitted to high volume inpatient Ebola Treatment Units (ETUs) over an eight-month period during the recent EVD epidemic in Eastern DRC. Patients with rhabdomyolysis were compared to those without. Laboratory measurements included: viral load (VL), creatine kinase (CK), and creatinine (Cr). Acute kidney injury (AKI) was defined according to Kidney Disease: Improving Global Outcomes (KDIGO) guidelines. 426 EVD patients were admitted to the ETUs in Butembo and Katwa between 30 March and 1 October, 2019. Ninety-three patients (22%) had no measurement of CK and were excluded. The remaining 333 patients had 2 229 CK measurements (median 5 [IQR 1-11] measurement per patient) over the course of the admission. Of these, 271 (81%) had an elevated CK (>380U/L), 202 (61%) patients had rhabdomyolysis (CK>1000 IU/L), and 45 (14%) had severe rhabdomyolysis (≥5 000U/L). The longitudinal profile for CK differed significantly between survivors and non-survivors. Rhabdomyolysis was associated with acute kidney injury (AKI, OR 3.1 [95%CI 1.9-5.2], p<0.0001) and remained an independent predictor of AKI in a multivariable logistic regression model adjusting for confounding effects of VL (aOR 1.8 [95%CI 1-3.3], p=0.045). Rhabdomyolysis at admission predicted subsequent mortality (HR 3.1 [95%CI 2.1-4.7], p<0.0001) and remained a statistically significant independent predictor of mortality in a Cox proportional hazard model adjusting for other risk factors for mortality, including VL and AKI (aHR 1.7 [95%CI 1.1-2.9], p=0.034). In conclusion, rhabdomyolysis is associated with AKI and is an independent risk factor for mortality in EVD patients.

1537

RE-EMERGENCE OF CHAPARE HEMORRHAGIC FEVER IN BOLIVIA, 2019

Caitlin Cossaboom[1], Armando Medina[2], Carla Romero[2], Maria Morales-Betoulle[1], Gricel Alarcon[2], Jhemis Molina[2], Roxana Loayza[3], Cinthia Avila[3], Sebastian Sasias[3], Mirian Cruz[3], Eliana Gil[3], Gabriela Anez[3], Jimmy revollo[3], Fernando Morales[3], Carlos E. Alvarez[3], Jairo Mendez-Rico[4], Shannon Whitmer[1], Ketan Patel[1], John Klena[1], Stuart Nichol[1], Christina Spiropoulou[1], Mary Choi[1], Trevor Shoemaker[1], Joel Montgomery[1]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Unidad de Epidemiología, Ministerio de Salud, La Paz, Plurinational State of Bolivia, [3]Centro Nacional de Enfermedades Tropicales, Santa Cruz de la Sierra, Plurinational State of Bolivia, [4]Pan American Health Organization, Washington, DC, United States*

Chapare virus (CHAPV), causative agent of Chapare hemorrhagic fever (CHHF), is a rodent-borne virus (genus *Mammarenvirus,* family *Arenaviridae*). The only previously confirmed case of CHHF was a 2004 fatality in an agricultural worker in Cochabamba, Bolivia with limited epidemiological information. In June 2019, the Bolivian Ministry of Health reported a hemorrhagic fever cluster of unknown etiology. Case one, 65-year-old agricultural worker, presented in Caranavi on 1 May reporting eight days of fever, myalgia, retro-orbital pain, abdominal pain, and vomiting progressing to include gingival hemorrhage. He died on 12 May, suspected of severe dengue. Case two, 25-year-old medical intern, attended to case one on 11 May, developed identical symptoms on 20 May, was transferred to La Paz on 2 June, and died two days later. Case three, 22-year-old agricultural worker, spent the night in the hospital with case one on 11 May and developed identical symptoms with maculo-papular rash and irritability on 29 May. On 3 June, he was transferred to La Paz and discharged on 30 June. Two healthcare workers, cases four and five, had contact with case two during transfer on 2 June and endoscopy on 4 June, and developed fever, arthromyalgia, and malaise on 18 June that progressed to gingival hemorrhage. Case four, 48-year-old ambulance worker, eventually recovered, while case five, 42-year-old gastroenterologist, died on 10 July. Specimens from two cases were shipped to the Centers for Disease Control and Prevention in Atlanta, USA where we identified a novel strain of Chapare virus. A real-time RT-PCR assay was developed that detected CHAPV RNA in different specimens from four cases. Case one remains probable as no specimens were available. CHAPV RNA was detected in blood, saliva, urine, and semen of the two survivors following recovery. We report a confirmed outbreak of CHHF in Bolivia. Novel disease characteristics are described including human-to-human transmission and viral persistence in bodily fluids following recovery. This investigation highlights the need for enhanced surveillance, public awareness, and improved identification and prevention of CHHF.

1538

OPERATIONALIZING GENOMIC EPIDEMIOLOGY DURING THE NORD-KIVU EBOLA OUTBREAK, DEMOCRATIC REPUBLIC OF THE CONGO

Eddy Kinganda Lusamaki[1], Allison Black[2], Daniel Mukadi[1], James Hadfield[3], Placide Mbala Kingebeni[1], Catherine Pratt[4], Adrienne Aziza Amuri[1], Junior Bulabula[1], Fabrice Mambu[1], Michael Wiley[4], Steve Ahuka Mundeke[1], Trevor Bedford[3], Jean Jacques Tamfum Muyembe[1]

[1]*Institut National de Recherche Biomediacle, Kinshasa, Democratic Republic of the Congo,* [2]*University of Washington, Washington, WA, United States,* [3]*Fred Hutch, Seattle, WA, United States,* [4]*University of Nebraska Medical Center, Omaha, NE, United States*

The Democratic Republic of the Congo declared its tenth Ebola virus disease outbreak in July 2018, which has circulated primarily in the Nord Kivu province. In addition to standard epidemiologic surveillance and response efforts, the Institut National de Recherche Biomédicale implemented an end-to-end genomic surveillance system, including sequencing, bioinformatic analysis, and dissemination of genomic epidemiologic results to frontline public health workers. As of February 2020 we have sequenced 586 genomes, representing 17% of all laboratory-confirmed infections. To support outbreak response efforts, we reconstructed spatiotemporal transmission dynamics at broad and at fine scales as new data were available and disseminated these results via an interactive narrative-based platform. We present a phylodynamic analysis of the outbreak, describing the types of epidemiologic dynamics that we monitor the genomic data for, including resolution of co-circulating transmission chains, detection of superspreading events, inference of regions that act as transmission sources and sinks, and differentiation of closely linked cases versus propagated transmission. Our innovative system enables sharing of these inferences between scientists and epidemiologists coordinating the day-to-day response on the time scales necessary to guide response efforts. The development of this genomic surveillance pipeline, within a resource-limited setting, represents significant technological and scientific progress in genomic epidemiology. These findings have ameliorated the current outbreak response and are directly applicable to future outbreaks.

1539

MALNUTRITION IS STRONGLY ASSOCIATED WITH CHILDHOOD MORTALITY IN SUB-SAHARAN AFRICA AND SOUTH ASIA: FINDINGS FROM THE CHILD HEALTH AND MORTALITY PREVENTION SURVEILLANCE (CHAMPS) NETWORK

Kasthuri Sivalogan[1], Priya M. Gupta[1], Ashka Mehta[2], Nega Assefa[3], Shams El Arifeen[4], Victor Akelo[5], Quique Bassat[6], Beth A. Tippett Barr[5], Emily Gurley[7], Amara Jambai[8], Shabir A. Madhi[9], Lola Madrid Castillo[10], Inacio Mandomando[11], Portia C. Mutevedzi[9], Ikechukwu U. Ogbuanu[12], Dickens Onyango[13], Anthony Scott[10], Samba Sow[14], Karen Kotloff[2], Parminder Suchdev[1], Dianna M. Blau[15]

[1]*Emory University, Atlanta, GA, United States,* [2]*University of Maryland Baltimore, Baltimore, MD, United States,* [3]*Haramaya University, Harar, Ethiopia,* [4]*icddr,b, Dhaka, Bangladesh,* [5]*CDC, Kisumu, Kenya,* [6]*ISGlobal, Barcelona, Spain,* [7]*John Hopkins University, Baltimore, MD, United States,* [8]*Sierra Leone MoHS, Freetown, Sierra Leone,* [9]*Wits University, Johannesburg, South Africa,* [10]*London School of Tropical Medicine & Hygiene, London, United Kingdom,* [11]*Centro de Investigação em Saúde de Manhiça, Manhica, Mozambique,* [12]*Crown Agents, Freetown, Sierra Leone,* [13]*Kisumu County Ministry of Health, Kisumu, Kenya,* [14]*Centre Pour les Vaccins en Développement, Bamako, Mali,* [15]*CDC, Atlanta, GA, United States*

Although WHO estimates ~50% of under-5 deaths are associated with malnutrition, no systematic processes exist to assess the contribution of malnutrition to under-5 mortality. Further, the *International Classification of Diseases* primarily categorizes the degree of malnutrition based on weight measurements or when unavailable, using relevant clinical evidence. CHAMPS is a surveillance platform that defines and assigns causes of stillbirth and under-5 deaths in seven sites. Local panels analyze available case information – including postmortem anthropometric measurements and photographs obtained from the minimally invasive tissue sampling procedure, histopathology results, clinical records, and verbal autopsy – to systematically assign underlying, immediate and contributing causes of death (CoD). Weight-for-length z-scores (WLZ) are calculated based on WHO growth standards to assess moderate-to-severe acute malnutrition or wasting, defined as WLZ<-2. WLZ were calculated for 698 cases 0-59 months including 282 neonates (0-27 days), 219 infants (28 days to <12 months) and 197 children (12 months to <60 months) at time of death. Among these cases, 81.7% (570/698) were classified as wasted and among cases classified as wasted, 25.3% (144/570) listed malnutrition in the CoD. Of cases with malnutrition listed as the underlying CoD (n=82) or in the causal chain (n=26), 93.9% and 88.5% cases, respectively, were classified as wasted. In cases where malnutrition was listed as a significant condition contributing to death (n=36), 72.2% were classified as wasted. Due to growth standards limitations, further analysis, with adjustments for gestational age, is needed to interpret WLZ measurements for neonates and infants born small for gestational age. CHAMPS data highlights concordance between the prevalence of wasting and categorization of malnutrition in the CoD. Additional examination is necessary, especially when WLZ was indicative of wasting but malnutrition was not listed in the causal chain. Improved systematic assessment of malnutrition as a cause of under-5 mortality is necessary to inform nutrition programs in LMICs.

1540

EFFORTS TO IMPROVE POSTMORTEM ANTHROPOMETRIC MEASUREMENTS IN CHILDREN UNDER 5 YEARS OF AGE

Priya M. Gupta[1], Victor Akelo[2], O Yaw Addo[3], Kasthuri Sivalogan[4], Richard Oliech[5], Dickson Gethi[5], Beth Tippett Barr[6], Dianna Blau[7], Parminder Suchdev[3]

[1]*Emory University, Nutrition & Health Sciences Program, Atlanta, GA, United States,* [2]*US Centers for Disease Control and Prevention-Kenya, Kisumu and Nairobi, Kenya, Nairobi, Kenya,* [3]*Emory University, Nutrition & Health Sciences Program, Emory Global Health Institute, US Centers for Disease Control and Prevention, Atlanta, GA, United States,* [4]*Emory Global Health Institute, Atlanta, GA, United States,* [5]*Kenya Medical Research Institute, Nairobi, Kenya,* [6]*Emory University, Nutrition & Health Sciences Program, US Centers for Disease Control and Prevention-Kenya, Kisumu and Nairobi, Kenya, Nairobi, Kenya,* [7]*US Centers for Disease Control and Prevention, Atlanta, GA, United States*

The Child Health and Mortality Prevention Surveillance Network (CHAMPS) aims to identify causes of under-5 mortality in sub-Saharan African and South Asia. To address challenges in postmortem nutritional assessment, we evaluated anthropometry training and 3D imaging in the CHAMPS Kenya site. Staff were trained using WHO recommended standard anthropometry (SA) equipment and novel 3D imaging methods to collect postmortem measurements. Following the training, 76 deaths were measured in duplicate and were compared to 75 pre-intervention deaths. Data quality metrics included standard deviations (SD), digit preference (DP), % biologically implausible values (BIV), technical errors of measurement (TEM), correlation coefficients, and Bland Altman plots. We used both WHO growth standards and internal standardization to produce length-for-age z scores (LAZ). Lastly, we analyzed median differences between pre- and post-mortem height measurements. Standard anthropometry data quality improved after training, as indicated by DP. When using the WHO growth standards, we observed increases between pre- and post-training LAZ SD (2.6 vs. 2.9) and % BIV (5.3 vs. 15.8). Internal standardization eliminated BIV, with LAZ ranging from -1.8 to 2.3 pre-intervention and 2.3 to 2.0 post intervention, falling within WHO ranges for biologically plausible values (-6 SD<LAZ<6 SD). Reliability of length measurements post-intervention was high as indicated by low relative TEM of 0.53%. 3D imaging was highly correlated with SA measurements (R=0.99) measurements for length; however, on average

3D scans overestimated length by 3.8 cm. Pre-mortem height data was available on 17% of cases, and median differences between pre- and post-mortem heights among those who did and did not participate in the intervention was -1.5 cm and -2.0 cm, respectively. Training on SA improved data quality. 3D imaging may be an accurate alternative to SA, but adjustment of the technology is needed to avoid overestimation of length. Future research on the appropriate use of reference standards to define malnutrition in this severely ill population is needed.

1541

TRAINING MOTHERS IN THE DR CONGO IN EARLY CHILDHOOD DEVELOPMENT TECHNIQUES TO MITIGATE THE RISK OF EARLY CHILDHOOD NEURODEVELOPMENTAL DEFICITS FROM DEPENDENCE ON POORLY PROCESSED TOXIC CASSAVA

Michael J. Boivin[1], Esperance Kashala-Abotnes[2], Alla Sikorskii[1], Nicole Mashukano[3], Marcel L. Kunyu[4], Daniel Mukeba[4], Miriam Namirembe[5], Fiona Bukenya[5], Daniel Okitundu[4], Dieudonne Ngoyi Mumba[6], Desire Tshala-Katumbay[7]

[1]*Michigan State University, East Lansing, MI, United States,* [2]*University of Bergen, Bergen, Norway,* [3]*Programme National de Nutrition (PRONANUT), Kinshasa, Democratic Republic of the Congo,* [4]*University of Kinshasa, Kinshasa, Democratic Republic of the Congo,* [5]*Global Health Uganda, Kampala, Uganda,* [6]*Institute Nationale de Recherche Biomédicale (INRB), Kinshasa, Democratic Republic of the Congo,* [7]*Oregon Health and Sciences University, Portland, OR, United States*

Boivin and colleagues established the efficacy of year-long mediational intervention of sensitizing caregivers (MISC) training program for early child development (ECD) in rural Uganda with HIV mothers. Such a program with mothers of konzo-affected households in the DR Congo might help their preschool-age children in the face of konzo disease from poorly processed toxic cassava. Konzo disease is an irreversible upper-motor neuron disease affecting mostly women and children in regions subsisting on poorly processed bitter cassava high in glucogenic cyanide. Our group was the first to document neurodevelopmental delay of infants/toddlers in this region. Randomized controlled trial (RCT) for a year of biweekly MISC training was embedded into a non-inferiority trial of training mothers in the wetting method (WTM) for the safe processing of bitter cassava. The study took place in the Kahemba health zone of the province of Bandundu DRC, with the highest global prevalence of konzo disease. Households with pre-school children were randomized to MISC ECD (N=96 households) or control (N=138) while balancing on the WTM trial arm assignment. Linear mixed effects (LME) models were used to evaluate the main effects of the MISC trial arm at 6 and 12 months (average over time) on the Mullen Scales of Early Learning (MSEL) scales while adjusting for baseline value of each MSEL score, height-for-age z-score, HOME caregiving quality, and WTM trial arm assignment. The average MSEL cognitive composite score over time was higher in the MISC arm LSM=70 (standard error [SE]=1.73) than control LSM=65 (SE=1.85), which was clinically meaningful. Significant differences between trial arms were seen in three cognitive domains of the MSEL: fine motor scores (p=.006), receptive language (p=.028), and visual perception (p=.029). Our MISC program was the first time such an intervention has been used to mitigate neurodevelopmental risk from toxic exposure of cyanide within poorly processed cassava. The public health potential is great given the reliance on toxic cassava throughout central/western Africa and poor ECD resources.

1542

THE ADDED VALUE OF THE MINIMALLY INVASIVE TISSUE SAMPLING OVER MEDICAL RECORDS AND VERBAL AUTOPSY IN DIAGNOSING THE CAUSE OF DEATH AMONG UNDER-5 CHILDREN AND STILLBIRTHS IN BANGLADESH

Farzana Islam[1], Kyu Han Lee[2], Atique Iqbal Chowdhury[1], Afruna Rahman[1], Abu Faisal Md Pervez[3], Abu Bakkar Siddique[1], Qazi Sadeq-ur Rahman[1], Kazi Munisul Islam[1], Syed Aminul Islam[1], Shovo Debnath[1], Sharmin Sultana[1], Rajib Biswas[1], Faria Ahmed[1], Sabbir Ahmed[1], Muntasir Alam[1], Mustafizur Rahman[1], Sanwarul Bari[1], Shams El Arifeen[1], Emily S. Gurley[2]

[1]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh,* [2]*John Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [3]*Faridpur Medical College Hospital, Faridpur, Bangladesh*

In Bangladesh, cause of death is determined through clinical diagnosis prior to death and/or by verbal autopsy (VA). These methods are limited by lack of diagnostic information, substantial recall bias, and inability to distinguish between diseases with similar clinical presentations. Child Health and Mortality Prevention Surveillance (CHAMPS) network has been conducting minimally invasive tissue sampling (MITS) to systematically describe causes of under-5 child death and stillbirth in Bangladesh. Since September 2017, we identified under-5 children and pregnant women admitted to 3 hospitals in 2 districts. For deaths and stillbirths, with consent from guardians, we conducted histopathology, immunohistochemistry, multi-pathogen molecular based assays, and microbiological culture on postmortem specimens, as well as VA interviews. A specialized panel reviewed all clinical, VA, and MITS data to determine a definitive cause of death for 69 under-5 deaths and 56 stillbirths. To identify the value-added of MITS beyond clinical and VA diagnosis, we compared these findings with clinical diagnoses and InterVA5, a probabilistic algorithm-based assignment of cause of death using VA data. Among 125 MITS cases, no clinical diagnosis had been made for 57 cases (46%), whereas, 100% were diagnosed through MITS. Sepsis was identified as cause of death almost three times more often in MITS than clinical diagnosis (15% vs 6%). The cause of death was unspecified in 21 cases of 69 under-5 deaths (30%) when using VA reports alone. Clinical data could not be used to determine a cause in 53 of 56 stillbirths (95%); VA findings showed only broad stages of macerations and could not identify a specific cause of death for any case. However, MITS identified a cause in 100% cases and the causes were mainly preterm and low birth weight, intrauterine hypoxia/birth asphyxia, sepsis and congenital anomaly. MITS procedure provides substantial improvements in identifying causes of child death and stillbirths over clinical diagnoses or VA alone. The cause of deaths determined through MITS could help to prioritize interventions to reduce under-5 mortality and stillbirths.

1543

SOCIAL INTEGRATION AMONG PRIMIPAROUS WOMEN IN RURAL BANGLADESH AND ASSOCIATIONS WITH PERINATAL DEATH

Kyu Han Lee[1], Atique I. Chowdhury[2], Shahana Parveen[2], Qazi S. Rahman[2], Mamunur Rashid[2], Solveig A. Cunningham[3], Sanwarul Bari[2], Shams El Arifeen[2]

[1]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [2]*International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh,* [3]*Emory University, Atlanta, GA, United States*

Social integration - defined as the extent of social ties and connections an individual has access to - plays a major role in disease risk, disabilities and mortality. In this study, we explored whether social integration might influence maternal and child health by using in-migration as a proxy for limited social integration among newly married women in rural Bangladesh. We used data from an ongoing demographic surveillance system - a part of the Child Health and Mortality Prevention (CHAMPS)

network - to identify primiparous women in Baliakandi, a rural sub-district of Bangladesh who were newly married between September 2017 and October 2019. We restricted our analysis to women who had their first birth at 20-24 years of age and defined recent in-migration as migration into Baliakandi sub-district within 12 months of marriage. We used logistic regression to estimate the association between recent in-migration and perinatal deaths (stillbirths and deaths within 7 days of birth), after adjusting for household wealth and mother's age. Among 316 first births among newly married women, 127 (40%) were among women who migrated within 12 months of marriage; 101 in-migrations occurred within 1 month of marriage (80%). Five of the total 12 stillbirths (42%) and 10 of the total 14 early neonatal deaths (71%) were among recent migrants. The adjusted odds of early neonatal deaths were significantly higher (OR: 3.8, 95% CI: 1.1-12.6) among recent migrants compared to other newly married women. We did not find a statistically significant association with stillbirths (OR: 1.0, 95% CI: 0.3-3.3). Further investigations are needed to identify causal mechanisms between recent migration and early neonatal deaths, particularly sociocultural factors, and to determine how we might improve the health of these women and their newborns.

1544

ASSESSMENT OF THE REPRODUCTIVE AND SEXUAL HEALTH OF THE ADOLESCENT POPULATION FROM TEN RURAL COMMUNITIES IN THE SIERRA MADRE REGION IN CHIAPAS (MEXICO)

Zeus Aranda Remon, **Melania Muñoz Ramírez**, Hellen Mata González

Compañeros En Salud (Partners In Health Mexico), Jaltenango de la Paz, Mexico

In 2017, Partners In Health Mexico, a non-governmental organization founded in the Sierra Madre region in Chiapas, Mexico, in 2012, implemented a maternal health support program in eleven rural clinics, with the aim of providing complete, timely and adequate antenatal care (ANC), childbirth care and postpartum care to the women living in the Sierra. After two years running the program, we observed an important flow of adolescents (10 to 19 years of age) reaching the clinics. Adolescent pregnancy increases the risk of complications during pregnancy, low weight of the newborn and infant mortality. Moreover, the usage of ANC and postpartum care is less common among adolescents. We decided to conduct a situational analysis of the reproductive and sexual health in women under 20 years in the ten communities where we collaborate. We collected 2019 data from the electronic medical records of the eleven rural clinics. Additionally, we conducted a survey in two of the ten rural communities in January 2020. In 2019, 24% of 442 pregnant women that attended the rural clinics were under 20 years old. 72% of the adolescents that delivered in the second semester of 2019 had followed a complete antenatal control (5 visits or more; 63% in the overall population), 44% had sought ANC in the clinic for the first time in the first quarter of pregnancy (44% in the overall population) and 75% had visited the clinic in the 15 days after giving birth (73% in the overall population). In the two communities where the survey was conducted, 22% of 119 women under the age of 20 had become sexually active, including no women under the age of 15. The unmet demand for modern contraceptives in women under 20 in union or married (n = 17) was 29% and in the overall population in reproductive age in union or married (n = 335) was 21%. After the analysis, we identified the following priorities: to strengthen family planning counseling services for the adolescent population and to invest in the retention of the overall population of pregnant women in the continuum of maternal care, since the age does not seem to have an impact on maternal healthcare services utilization.

1545

ORAL REHYDRATION THERAPIES IN SENEGAL, MALI, AND SIERRA LEONE: A SPATIAL ANALYSIS OF CHANGES OVER TIME AND IMPLICATIONS FOR POLICY

Kirsten E. Wiens, Lauren E. Schaeffer, Brigette F. Blacker, Simon I. Hay, Robert C. Reiner, Jr

Institute for Health Metrics and Evaluation, University of Washington, Seattle, WA, United States

Oral rehydration solution (ORS) is a simple intervention that can prevent childhood deaths from severe diarrhea. In a previous study, we mapped ORS subnationally and found that ORS coverage increased over time, while use of home-made alternatives or recommended home fluids (RHF) decreased, within Senegal, Mali, and Sierra Leone. It was unclear, however, whether ORS replaced RHF in these locations or if children were left untreated, and if these patterns were associated with health policy changes. Understanding the basis for such changes in treatment is critical to ending preventable child deaths from diarrhea. Therefore, the goal of this study was to examine whether RHF was replaced with ORS before and after interventions, policies, and external events that may have impacted healthcare access in these countries. We used a Bayesian geostatistical model and data from household surveys to map the percent of children with diarrhea that received (1) any ORS, (2) only RHF, or (3) no oral rehydration treatment between 2000 and 2018. In addition, we conducted a literature review to identify key events that impacted healthcare access, and discussed the findings with in-country experts. We found subnational locations within Mali and Senegal where increases in ORS were smaller than declines in RHF, suggesting that children had been left behind during efforts to scale up ORS treatment. In contrast, we found that a national-level policy that abolished health costs for pregnant women, new mothers, and children in Sierra Leone was associated with increases in ORS coverage. These findings suggest that policies to promote ORS use need to be accompanied by efforts to make ORS affordable and widely available. Furthermore, we show that events such as conflict and the Ebola outbreak in Sierra Leone likely negatively impact ORS access. Effective messaging regarding appropriate diarrhea treatment could save child lives during these destabilizing events, as well as in locations where ORS has not yet replaced RHF. These expanded efforts will be critical to reach the over 150 000 children with diarrhea that do not receive ORS in Mali and Senegal.

1546

DEVELOPMENT A MULTIPLEX BEAD ASSAY FOR DETECTION OF ANTIBODIES AGAINST *TRYPANOSOMA CRUZI* CHRONIC INFECTION

Sukwan Handali, Yong Wang, Olga Stuchlik, Matthew S. Reed, Pavel Svoboda, Hilda N. Rivera, Katie Bowden, Ryan E. Wiegand, Diana L. Martin, Jan Pohl

CDC, Atlanta, GA, United States

Chagas disease is a parasitic infection caused by the protozoan parasite *Trypanosoma cruzi,* which is transmitted to animals and people by triatomid bug vectors. Acute infection, typically the first 4-8 weeks, is diagnosed through direct detection of parasites or parasite DNA. During the chronic phase of infection, which can be life-long, parasites reside primarily in muscle tissue and are not easily found in the bloodstream. Therefore, diagnosis of chronic infection is through detection of circulating *T. cruzi*-specific IgG antibodies. However, because *T. cruzi* has a high genetic and phenotypic intraspecies diversity there is variation in host antibody responses that can make serologic diagnosis challenging. We therefore attempted to identify novel antigen targets for use in a multiplex-based assay (MBA) to detect antibodies against multiple defined targets. We separated crude antigens of *T. cruzi* using size exclusion fast-performing liquid chromatography followed by 1-D gel electrophoresis and then Western blot to find the reactive bands. These bands were then subjected to tandem mass spectrometry. These approaches avoided the use of 2-D gel electrophoresis that is technically challenging and has reproducibility issues. From 2187 protein sequences, based on selection

criteria (such as size, presence of transmembrane domain, and availability of signal peptide sequences), 37 unique proteins were expressed, purified, and tested for reactivity against positive sera on MBA. Six of these showed a signal-to-noise ratio greater than 5 in an MBA; these were subsequently tested on an MBA with a serum panel of 76 defined negative, 227 defined positive chronic Chagas, and 126 defined positive *Leishmania* spp. from Chagas endemic countries. Using defined negative and positive sera, three antigens showed sensitivities of 92-96% and specificity of 98-99%. The 3 other antigens had sensitivities of 71 - 89% and specificities of 65-91%. Cross-reactivities to *Leishmania* spp. positive sera are in the range of 15-33%. In conclusion, using a new approach of not using 2D-gel electrophoresis, we found 3 diagnostic markers that perform well in the MBA.

1547

REVIEW OF CHAGAS DISEASE DIAGNOSTIC TESTING AT A MAJOR UNIVERSITY HOSPITAL IN CALIFORNIA

Emily A. Kelly, Caryn Bern, Jeffrey D. Whitman

University of California, San Francisco, San Francisco, CA, United States

Chagas disease (CD) is a neglected parasitic disease endemic to Latin America. Transmission of the etiologic agent, *Trypanosoma cruzi*, is predominantly vector-borne, and established infection can persist lifelong in the absence of treatment. In the United States, the majority of CD cases are imported, and there is a lack of awareness around screening and diagnosing of this disease. In order to capture CD diagnostic testing practices, researchers extracted electronic chart data on all patients tested for CD at the University of California, San Francisco, between February, 17th, 2016, and December 19th, 2019. Demographic data, test information, indication and potential risk factors were reviewed. This study was approved by institutional review board at UCSF. During the study period, 125 combination IgG and IgM serological assays were performed on specimens from 109 patients. Eleven (11/125, 9%) tests were reactive for anti-*T. cruzi* antibodies, 5 sera were sent to CDC for confirmation, and 3 (3/109, 3%) new CD diagnoses were made. All three patients diagnosed were born in Latin America. The median age of patients tested was 52 years [interquartile range: 37, 63] and 70 (64%) were men. 37 (34%) patients identified as Hispanic non-white and 40 (37%) as non-Hispanic white. Among non-Hispanic white patients, short term travel to Latin America was the most commonly identified risk factor (55%). Among 10 patients with countries of birth outside of the Americas (7 in Asia, 2 in Africa, 1 in the Middle East), none had documented travel to Latin America. The three specialties most likely to order CD testing were cardiology (63, 50%), hematology/oncology (13, 10%) and cardiothoracic surgery (10, 8%). No diagnostic testing was identified from OB/GYN services. During the period studied, CD testing was conducted predominantly in non-Hispanic white patients. CD diagnosis occurred exclusively in Hispanic patients born in Latin America. No pre- or post-natal testing was undertaken despite potential for congenital transmission of CD. Although limited in scope, our analysis illustrates important gaps in CD diagnostic testing.

1548

THE DIAGNOSTIC CAPABILITIES OF AN IN-HOUSE ELISA METHOD IN THE DIAGNOSIS OF CUTANEOUS LEISHMANIASIS CAUSED BY *LEISHMANIA DONOVANI* IN HAMBANTOTA DISTRICT SRI LANKA

N. L. De Silva[1], V. N. De Silva[2], A. T. Deerasinghe[3], H. Kato[4], M. Itoh[5], H. Takagi[5], M. V. Weerasooriya[1], T. C. Yahathugoda[1]

[1]*Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka,* [2]*Base Hospital Tangalle, Tangalle, Sri Lanka,* [3]*District General Hospital Hambantota, Hambantota, Sri Lanka,* [4]*Jichi Medical University, Tochigi, Japan,* [5]*Aichi Medical University School of Medicine, Aichi, Japan*

Clinical diagnosis has become a challenge amidst a surge of cutaneous leishmaniasis in Southern Sri Lanka. Slit-skin smear (SSS) to confirm its diagnosis has only 78% sensitivity. We developed a new in-house ELISA method and assessed its diagnostic capabilities against PCR (gold-standard - Gs). Sensitivity of the modified nested PCR was tested using serial dilutions (10^3 to 10^{-2} parasites) of DNA extract of cultured *L. donovani* DD8 strain. ITS region amplification was performed using outer primers—LITSR: 5′-CTGGATCATTTTCCGATG-3′ and L5.8S: 5′-TGATACCACTTATCGCACTT-3′— and two novel inner primers—LITSR-inner: 5′-CATTTTCCGATGATTACACC-3′ and L5.8S-inner: 5′-TACTGCGTTCTTCAACGA-3′. A cohort of 194 PCR positives was examined by SSS microscopy by hospital technicians and authors, by rK39-ICT and by an in-house IgG-based ELISA method using rKRP42 recombinant antigen. Validation was done using non-endemic sera and cutoffs developed using ROC. The diagnostic capability was expressed by means of sensitivity (Sn), specificity (Sp), Positive & Negative Predictive Values (PPP, NPP) and Kappa inter-rater agreement test (Kp). The PCR was sensitive enough to detect 10^{-1} parasites. SSS by technician vs. Gs (Sn=77.6%; Sp=45.5; PPV=95.9; NPP 10.9%; Kp=0.09/p=0.08). SSS by authors vs. Gs (Sn=80.9%; Sp=81.8; PPV=98.7; NPP 20.5%; Kp=0.26/p<0.01). ELISA vs. Gs (Sn=94.4%; Sp=50.0%; PPV=97.1; NPP 33.3%; Kp=0.39/p<0.01). Nine of 100 Gs became positive by rK39-ICT, and recorded very high antibody titers by ELISA. Combination of SSS (by technician) & ELISA vs. Gs (Sn=98.9%; Sp=30.0; PPV=96.2; NPP 60.0%; Kp=0.378/p<0.01). Higher diagnostic accuracy of Gs allows detection of lesions with low parasite densities missed by SSS. However, the diagnostic accuracy can be increased by screening of SSS by an expert or combine SSS (by technician) and ELISA. Advanced studies are required to understand the higher anti-rKRP42 antibody titers and positives for rK39-ICT.

1549

PRECLINICAL DRUG CANDIDATES FOR CHAGAS DISEASE TARGETING THE *TRYPANOSOMA CRUZI* METHIONYL-TRNA SYNTHETASE

Frederick S. Buckner, Aisha Mushtaq, John R. Gillespie, Nora R. Molasky, Aleksander C. Lazarski, Zhongsheng Zhang, Wenlin Huang, Sayaka Shibata, Erkang Fan

University of Washington, Seattle, WA, United States

Trypanosoma cruzi infection afflicts 6-8 million people primarily in Latin America. The current antiparasitic drugs for Chagas disease are inadequate due to numerous side effects and poor efficacy in chronically infected patients. The *T. cruzi* has a single methionyl-tRNA synthetase (MetRS) which is essential for protein synthesis. Our work is targeting the MetRS enzyme with novel inhibitors to develop leads for anti-*T. cruzi* drug development. The MetRS inhibitors were developed against the *Trypanosoma brucei* MetRS guided by crystal structure of that enzyme bound to inhibitors. Over 500 compounds from a library of MetRS inhibitors have been screened against mammalian-stage *T. cruzi* cultures establishing structure activity relationships. Over 70 compounds have been identified with EC_{50} values less than 10 nM and another 96 in the range of 11-100 nM. Most compounds are highly selective with cytotoxicity (CC_{50}) values on mammalian cells of greater than 1,000 nM. An exemplary compound was tested in an *in vitro* washout assay (16-day exposure at 25X the EC_{50}) and shown to have trypanocidal activity (with no outgrowth in the 60 day observation period), comparable to the clinical drug, benznidazole. The active compounds represent several distinct scaffolds within the MetRS inhibitor series and are associated with different physicochemical (e.g. LogP, solubility) and pharmacological properties. A subset of compounds is being tested in the murine chronic *T. cruzi* infection model, with results to be presented. New compounds have observed to have a high volume of distribution, a feature believed to be favorable for treating this tissue-based parasitic infection. MetRS inhibitors represent a promising class of compounds for treating Chagas disease.

1550

EFLORNITHINE ANTITRYPANOSOMAL EFFECTS ELICITED BY ITS L-STEREOISOMER *IN VITRO*

Mikael Boberg[1], Monica Cal[2], Marcel Kaiser[2], Rasmus Jansson-Löfmark[3], Pascal Mäser[2], Michael Ashton[1]

[1]*Unit for Pharmacokinetics and Drug Metabolism, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden,* [2]*Parasite Chemotherapy Unit, Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute & University of Basel, Basel, Switzerland,* [3]*DMPK, Research and Early Development Cardiovascular, Renal and Metabolism, BioPharmaceuticals R&D, AstraZeneca, Gothenburg, Sweden*

Repeated intravenous infusions of racemic eflornithine is since 1990 the recommended treatment for late stage Gambian Human African Trypanosomiasis (HAT). An oral eflornithine alternative would enhance treatment availability for patients in affected rural areas in sub-Saharan Africa. Attempts to treat with oral racemic eflornithine were unsuccessful due to dose-limiting gastrointestinal side effects or inadequate bioavailability. L-eflornithine has higher affinity to the target ornithine decarboxylase (ODC) compared to D-eflornithine in a cell free assay. With a formulation only containing the L-enantiomer, it is conceivable that therapeutic systemic exposure could be obtained at doses below the maximum tolerated oral racemic dose. The aim of this study was to investigate, for the first time, the enantioselective *in vitro* activity of eflornithine against *Trypanosoma brucei (T.b.) gambiense*. Eflornithine was donated by WHO/World Bank TDR, Geneva. D- and L-eflornithine were separated in house by chromatography. The drugs were dissolved in sterile water and diluted in HMI-9 medium before incubation with the *T.b. gambiense* strains STIB930, K03048 or 130R. The AlamarBlue serial drug dilution assay with a SpectraMax Gemini XS microplate fluorescence scanner was used. Modelling of the obtained data was performed in Phoenix (Version 8.2, Certara, Princeton, USA) to determine estimates for IC_{50}, I_{max} and the Hill factor. The IC_{50} value of L-eflornithine at 5.5 µM (95% confidence interval [4.8; 6.2 µM]) was significantly lower than that of D-eflornithine at 49.6 µM (41.7; 57.6 µM). As anticipated, the IC_{50} for racemic eflornithine was 9.1 µM (7.6; 10.6 µM), i.e. the higher potency of L-eflornithine was diluted by D-eflornithine. This suggests that primarily the L-enantiomer elicits the antitrypanosomal effect in the currently used racemic eflornithine clinical treatment regimen against HAT. Additionally, these findings justify further *in vitro* and *in vivo* studies to assess the feasibility for L-eflornithine as an oral treatment for late stage HAT.

1553

CONSTRUCTING A PHENOME: INTEGRATED ANALYSIS OF DRUG RESISTANCE, COMPETITIVE GROWTH AND GENE EXPRESSION IN NOVEL *PLASMODIUM FALCIPARUM* GENETIC CROSSES

Katrina A. Button-Simons[1], Sudhir Kumar[2], Katelyn M. Vendrely[1], Lisa A. Checkley[1], Mackenzie A. Sievert[1], Gabriel J. Foster[1], Catherine Jett[3], Xue Li[4], Douglas A. Shoue[1], Meseret T. Haile[2], Spencer Y. Kennedy[2], Ann Reyes[4], Abeer Sayeed[5], Marina McDew-White[4], François H. Nosten[6], Stefan H. Kappe[2], Scott J. Emrich[7], Timothy J. Anderson[4], Ian H. Cheeseman[3], Ashley M. Vaughan[2], Michael T. Ferdig[1]

[1]*Eck Institute for Global Health, Department of Biological Sciences, University of Notre Dame, St. Joseph, MI, United States,* [2]*Center for Global Infectious Disease Research, Seattle Children's Research Institute, Seattle, WA, United States,* [3]*Host-Pathogen Interactions Program, Texas Biomedical Research Institute, San Antonio, TX, United States,* [4]*Disease Intervention and Prevention Program, Texas Biomedical Research Institute, San Antonio, TX, United States,* [5]*Host-Pathogen Interactions Program and Disease Intervention and Prevention Program, Texas Biomedical Research Institute, San Antonio, TX, United States,* [6]*Shoklo Malaria Research Unit, Mahidol-Oxford Tropical Medicine Research Unit, Mahidol University, Mae Sot, Thailand,* [7]*Electrical Engineering and Computer Science, University of Tennessee, Knoxville, TN, United States*

Plasmodium falciparum genetic crosses have led to key discoveries of genes involved in many traits. Utilizing a human liver-chimeric mouse infused with human red blood cells (the FRG huHep/huRBC mouse), we can efficiently generate new crosses using recent patient-derived parasite isolates to capture the genetic basis of emerging drug resistance in real time. In the past two years we have produced 3 crosses to explore the basis of Art-R: 1) a clinically Art-R parasite that carries no mutation in K13; 2) an early C580Y K13 mutation from Thailand crossed with a sympatric parasite collected prior to the use of Art; and 3) a C580Y parasite from a lineage that is rapidly expanding across GMS crossed with a newly isolated susceptible African parasite. Quantitative trait locus (QTL) mapping can identify genetic loci and genes that contribute to phenotypes. This requires phenotyping large numbers of cloned progeny; our crosses have 84, 60, and 103 unique recombinant progeny. In parallel, we applied a powerful bulk segregant approach to identify selected loci by sequencing pooled uncloned progeny populations to measure changes in allele frequency. We developed a streamlined sampling protocol to simultaneously collect data for multiple phenotypes and generated an entire phenome in less than 3 months. We phenotyped subsets of these progeny for i) ring stage survival (RSA), ii) competitive growth as a surrogate for fitness in RBCs, iii) dose responses to four antimalarial compounds and, iv) genome-wide transcription profiles at three timepoints. This integrated data acquisition and analysis is designed to reveal how mutations can generate a range of resistance levels and fitness effects (i.e. compensation). By including genome-wide transcript profiles, we can interrogate a key intermediate step in the information cascade that connects genome variants to drug resistance and competitive growth phenotypes. Using a series of complimentary crosses can illuminate why some genetic backgrounds are more permissive for artemisinin resistance than others.

1554

UNRAVELING THE STRUCTURE AND FUNCTION OF THE NEMATODE SECRETORY SYSTEM TO IDENTIFY NEW ANTIFILARIAL TARGETS

Paul M. Airs, Zachary W. Heimark, Kendra Gallo, Kathy Vaccaro, Nicolas J. Wheeler, **Mostafa Zamanian**

University of Wisconsin-Madison, Madison, WI, United States

Excretory-secretory (ES) products released by parasitic nematodes into their host environment are considered essential for successful parasitism. There is active interest in resolving the composition of ES products, in exploring their interactions with host immune cells, and in mining secreted parasite molecules for diagnostic and helminthic therapy leads. However, despite the critical importance of ES processes, we have a superficial understanding of the underlying structure and function of the ES system in parasitic nematodes of importance to human medicine. ES systems are present throughout the phylum Nematoda but exhibit significant structural and functional diversity across clades and species. Recent studies in *Brugia malayi* suggest that the antifilarial action of ivermectin involves the inhibition of parasite secretory function. Building on these observations, we have taken steps to resolve the transcriptomic and structural states of the filarial nematode ES system using spatial (20 uM map) and single-cell RNA-seq approaches in *B. malayi*. These data were used to prioritize ES-associated receptors and ion channels belonging to druggable protein families and to identify candidate "hidden antigens" as vaccine targets. We hypothesize that prioritized receptors can be targeted to dysregulate the ES system in relevant stages and are therefore promising anthelmintic substrates. To this end, we have developed complementary whole-organism and target-based screening assays to profile parasite receptor pharmacology and to generate a better understanding of the relationship between apparent (motility) and cryptic (secretion) phenotypic states of filarial worms across intra-mammalian stages of parasitism. Together, these data and approaches outline a pathway to mining the critical interface of host-parasite communication for the discovery of new antifilarial targets.

1555

HUMAN PLACENTAL TROPHOBLASTS ARE RESISTANT TO *TRYPANOSOMA CRUZI* INFECTION IN A 3D CULTURE MODEL OF THE MATERNAL-FETAL INTERFACE

Erica Silberstein[1], Kwang Sik Kim[2], David Acosta[1], Alain Debrabant[1]

[1]*CBER/FDA, Silver Spring, MD, United States,* [2]*Johns Hopkins University School of Medicine, Baltimore, MD, United States*

Trypanosoma cruzi, the etiological agent of Chagas Disease (CD), is transmitted to humans by infected kissing bugs, blood transfusion, organ transplantation, and from mother to child. *T. cruzi* vertical transmission perpetuates CD and has become a globalized health problem accounting for 22% of new infections worldwide. Congenitally infected infants display low birth weight and although being generally asymptomatic, they may develop chronic CD with severe pathologies later in life. The human placenta forms a key barrier that protects the fetus from microbial infections. Interactions between *T. cruzi*, the placenta chorionic villi constituted by trophoblasts, and the maternal/fetal immune responses modulate the probability of vertical transmission. Yet, the mechanisms by which the parasite travels from the mother's blood stream to reach the developing fetus are unknown. Studies of congenital infections are challenging because they rely on the availability of human tissues, highly restricted by government regulations and ethical issues. To overcome these limitations, we employed a three-dimensional (3D) cell culture system to recreate the human placenta environment in which the trophoblast-derived JEG-3 cell line is co-cultured with human brain microvascular endothelial cells attached to beads in a rotating bioreactor. In this system, 3D-cultured JEG-3 (3D JEG-3) cells exhibit differentiation characteristics comparable to placental trophoblasts such as the formation of syncytia, and production of placenta specific hormones. Further, 3D JEG-3 cultures showed reduced susceptibility to *T. cruzi* infection compared to 2D JEG-3 cells (1% vs 20% of infected cells respectively), consistent with the intrinsic resistance of the human placenta to pathogen infections. To identify cellular factors and signaling pathways triggered in 3D JEG-3 cells during parasite infection, we performed gene expression and cytokine secretion profiling experiments. Our model may help to better understand the processes by which *T. cruzi* bypasses the human placental barrier and could be useful to evaluate therapeutics to reduce congenital CD.

1556

PATHOGEN BOX COMPOUNDS AS POSSIBLE LEADS FOR NEW INTERVENTIONS AGAINST LEISHMANIASIS

Wandayi Emmanuel Amlabu, Gordon A. Awandare, Theresa Manful Gwira

West African Center for Cell Biology and Infectious Diseases, WACCBIP, University of Ghana, East Legon, Accra, Ghana

Leishmaniasis is a disease closely associated with poverty and its endemic in Africa, Asia, southern Europe and the Americas. It is caused by parasites of the Genus *Leishmania* and transmitted by sandfly vectors. Its coinfection with HIV has increased its fatality wherever it's found and with the development of a vaccine against it still being a mirage, the most reliable intervention point against this disease remains chemotherapeutics. However, the current anti-leishmanial drugs are reported to be highly toxic, requires long term administration regimen, not readily accessible and costly. Here, we showed the anti-leishmanial activity *in vitro* and the likely mode of action of some 68 Medicine for Malaria Venture (MMV) compounds against the promastigotes and amastigotes stages of the *Leishmania donovani* parasites. The growth inhibitory concentrations (IC_{50}) obtained ranged between 10 nM and 95 µM. Twenty four (24) of the 68 compounds were tested for their cytotoxicity against RAW macrophage Cell lines and a selectivity index range of 0.03 to 455 was observed. The growth kinetic and growth reversibility profiles of twenty (20) of the compounds were indicative of a cytostatic effect, while another four (4) showed a cytocidal effect on the parasites, mediates programmed cell death through apoptosis and altered the cell cycle progression of the parasites by causing growth arrest at the G0-G1 and G2-M phases of the parasite cell mitotic division. Morphological analysis using fluorescence microscopy revealed obvious distortion in the mitochondrion integrity (60%) and the absence of DAPI-stained kinetoplasts DNA (30%). Our findings present useful therapeutic potentials of these compounds in Leishmaniasis.

1557

PHOSPHOMANNOMUTASE AS A NOVEL ANTIMALARIAL DRUG TARGET

Philip M. Frasse[1], Daniel Goldberg[1], Audrey R. Odom John[2]

[1]*Washington University in Saint Louis, Saint Louis, MO, United States,* [2]*Children's Hospital of Philadelphia, Philadelphia, PA, United States*

Malaria continues to pose an enormous economic and global health threat. With the constant barrier of antimalarial resistance and rising rates of delayed clearance by artemisinin, it is especially important to identify novel drug/target pairs with rapid antiparasitic activity. We study targetable metabolic pathways in the malaria parasite, *Plasmodium falciparum*, to guide such on-going drug development against this disease. In recent years, we have discovered that a large family of hydrolases, the Haloacid Dehalogenase (HAD) Superfamily of proteins, regulate a variety of *P. falciparum* metabolic pathways, which in turns leads to dramatic changes in central carbon metabolism and drug resistance. In this work, we investigate a related HAD protein, HAD5 (PF3D7_1017400), the putative phosphomannomutase that catalyzes the interconversion of mannose-6-phosphate and mannose-1-phosphate. This process is essential for mannose metabolism and parasite glycan synthesis, particularly for the synthesis of glycosylphosphatidylinositol (GPI) anchors. These GPI anchors are necessary to localize essential surface proteins in multiple parasite life stages, such as merozoite surface protein 1 (MSP1) and the vaccine target circumsporozoite protein (CSP). We have taken a multi-pronged approach to investigate HAD5 as an antimalarial target. First, we have validated recombinant HAD5 as a phosphomannomutase in biochemical assays. We have also generated transgenic parasites with regulated expression of HAD5 to demonstrate its essentiality and probe its effect on GPI synthesis and MSP1 localization. We have additionally obtained and solved a crystal structure of the enzyme, which differs significantly in surface charge from the human orthologs, showing promise for targetability. High throughput screening *in silico* is in process to identify candidate inhibitors with selective activity against the parasite enzyme. Thus, our studies validate and advance phosphomannomutase as an essential and druggable therapeutic target for future antimalarial development.

1558

IMPACTS OF IVERMECTIN-TREATED BACKYARD CHICKENS ON *CULEX* MOSQUITOES AND WEST NILE VIRUS TRANSMISSION IN DAVIS, CALIFORNIA

Karen M. Holcomb[1], Chilinh Nguyen[2], Brian D. Foy[2], Christopher M. Barker[1]

[1]*Davis Arbovirus Research and Training Lab, Department of Pathology, Microbiology, and Immunology, University of California, Davis, Davis, CA, United States,* [2]*Arthropod-borne and Infectious Diseases Laboratory, Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO, United States*

Current vector control strategies have limited ability to target the mosquitoes involved in enzootic and zoonotic transmission of West Nile virus (WNV) without disseminating pesticides over large areas. Ivermectin (IVM), a widely used antiparasitic drug in human and veterinary medicine, provides the potential for such control by targeting bird-feeding mosquitoes. Mosquitoes that ingest IVM experience increased mortality and few will survive long enough to take another bloodmeal. Thus, treating birds with IVM prevents future bites and thereby blocks subsequent transmission. We hypothesized that this would lead to targeted reduction in *Culex* vector indices and WNV transmission. To test this, we conducted a randomized trial in backyard chicken flocks in urban

neighborhoods across Davis, California. We placed eight flocks—four treated and four untreated control—of six chickens each in coops in backyards across Davis and weekly monitored entomological indices (i.e. abundance, WNV infection prevalence, and parity rate) near (10m) and far (150m) from each coop location for the WNV season (July-September 2019). We also monitored for WNV seroconversions in all chickens and IVM levels in the blood of treated chickens throughout the study. Fewer seroconversions occurred in treated flocks (3/17) compared to untreated flocks (11/24) and these occurred later in the season compared to untreated flocks, suggesting a lower WNV transmission at treated locations (p = 0.069). This work serves as a step toward development of novel WNV control strategies involving ivermectin delivered via passerine birds in residential neighborhoods.

1559

IDENTIFICATION OF MALARIA VECTORS AND INSECTICIDE RESISTANT USING MID-INFRARED SPECTROSCOPY THROUGH A PORTABLE QUANTUM CASCADE LASER DEVICE

Mauro Pazmino Betancourth, Francesco Baldini, Heather Ferguson, Klaas Wynne, Lisa Ranford-Cartwright, David Childs

University of Glasgow, Glasgow, United Kingdom

Vector-borne diseases are a major threat for public health, with 216 million cases of malaria worldwide. Recently, malaria management programmes have failed to continue to reduce the spread of disease, with an increment of 5 million cases in 2016. Parameters such as age, species and insecticide resistant are key to implementing and assessing these vector control programs. Despite this need, there are no reliable tools to gain this information suitable for widespread deployment. However, mid-infrared spectroscopy combined with data analysis can detect the fingerprint signatures of most molecules which allow us to classify biological samples. Unfortunately, whilst mid-infrared tools make for indispensable equipment in the modern well-funded laboratory, they have severe limitations in field applications. In this work, we evaluated the use of mid-infrared Fourier transform infrared diffuse reflectance (DRIFT) spectroscopy on female laboratory reared *Anopheles* mosquitoes for age, species and insecticide resistant classification. We employed linear discriminant analysis (LDA) and support vector machine (SVM) algorithms on spectra from insect legs. We successfully discriminated them into the two species and into to two age groups (3 days and 10 days old) and insecticide susceptibility with an accuracy of 80%, 75% and 73% respectively. In order to overcome the technical limitations of current mid-infrared tools, we have built an external cavity Quantum Cascade Laser system. The laser operates within the range of 9 -10 µm. We will detail the advantages of a semiconductor laser solution over FTIR and demonstrate its use as a spectroscopy system. We have conducted comparisons of QCL to FTIR with testing of polymer standards, through to mosquito tissues. Our results highlight the potential of mid-infrared spectroscopy as a tool for biological trait identification on mosquitoes. In addition, quantum cascade lasers offer a compact, cheaper option over current tools for spectroscopy. This can guide new ways of identifying mosquito traits that can lead the creation of surveillance programs with new technology into mosquito control tools.

1560

INSECTICIDE RESISTANCE ALTERS THE MICROBIOTA OF *ANOPHELES COLUZZII* FROM AGBOVILLE—A REGION WITH INTENSE PYRETHROID RESISTANCE IN CÔTE D'IVOIRE

Bethanie Pelloquin[1], Mojca Kristan[1], Constant Edi[2], Anne Meiwald[1], Emma Clark[1], Claire Jeffries[1], Thomas Walker[1], Nsa Dada[3], Louisa Messenger[1]

[1]*Faculty of Infectious Tropical Diseases, London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*Centre Suisse de Recherche Scientifique en Côte d'Ivoire, Abidjan, Côte D'Ivoire,* [3]*Faculty of Science and Technology, Norwegian University of Life Sciences, Aas, Norway*

Research on insecticide resistance mechanisms has intensified due to its increasing threat to malaria control efforts. Following evidence of links between the mosquito microbiota and insecticide resistance, with resulting significant enrichment of insecticide degrading bacteria and enzymes, we characterized and compared the microbiota of *Anopheles* (*An.*) *coluzzii* in relation to their deltamethrin resistance profiles. Non-blood fed, 2-3 day old (d), virgin female F_1 progeny of field caught *An. gambiae s.l.* from Agboville, Côte d'Ivoire underwent deltamethrin resistance phenotyping using modified CDC bottle bioassays, and subsequent molecular species identification. Mosquitoes identified as *An. coluzzii* were grouped, by resistance status: susceptible (died following exposure to the diagnostic dose of deltamethrin), resistant (survived exposure to 1, 5 or 10 times the diagnostic dose) or bioassay controls; and age at death: 2-3d (euthanised at 60 minutes post exposure) or 5-6d (euthanised at 72 hours post exposure) and processed as pools, comprised of 3 individuals each, with shared phenotypes and ages. Following Illumina *16S rRNA* amplicon sequencing using universal V3-V4 region primers, comparison of their microbiota revealed a significant reduction (q=0.003) in microbial diversity between 2-3d susceptible and resistant mosquitoes. There was also a significant reduction in microbial diversity between 2-3d and 5-6d mosquitoes overall (q=0.003), confirming reports that diversity decreases with age. *Acinetobacter, Corynebacterium* and *Sphingomonas*, each of which include insecticide degrading species, were significantly enriched in resistant mosquitoes. The results suggest these bacteria may contribute to, and/or that their enrichment is a consequence of, the observed resistance phenotypes. Our findings show significant alterations of *An. coluzzii* microbiota associated with deltamethrin resistance, corroborating results of studies on *Anopheles* species from other geographic locations and highlighting the potential for identification of novel microbial markers for insecticide resistance surveillance.

1561

SELECTION FOR INSECTICIDE RESISTANCE IN *ANOPHELES GAMBIAE* RESULTS IN INCREASED COMPETENCE FOR *PLASMODIUM FALCIPARUM* INFECTIONS

Kelsey L. Adams[1], Emily K. Selland[1], Naresh Singh[2], Flaminia Catteruccia[1]

[1]*Harvard T.H. Chan School of Public Health, Boston, MA, United States,* [2]*Harvard University, Boston, MA, United States*

Transmission of malaria by *Anopheles* mosquitoes has been reduced in many regions through the widespread use of Insecticide Treated Nets and Indoor Residual Spraying, but alarming intensities of insecticide resistance in many mosquito populations are threatening malaria control programmes. Many molecular mechanisms contribute to insecticide resistance, and it is critical to understand how these alterations affect *Plasmodium falciparum* development within the *Anopheles* vector. To date studies have only investigated the effect of target site mutations, so we endeavored to examine the effect of complex insecticide resistance on *P. falciparum* vector competence *in Anopheles gambiae s.l.* mosquitoes. From a parental cross between resistant (derived from VK5, Burkina Faso) and susceptible (G3) mosquitoes, we created both a highly resistant line (>90% survival to .75% permethrin) by routine permethrin exposure, and a susceptible line (0% survival to .75% permethrin) in which resistance was simply lost over time. Once generated, each colony was infected with *P. falciparum* to determine oocyst burden and sporozoite intensities. Despite largely sharing the same genetic background, permethrin resistant mosquitoes showed significantly higher infection intensities at the oocyst and sporozoite level compared to susceptible mosquitoes. While present at significant allele frequencies in the resistant mosquitoes, the *kdr* mutation was not associated with higher infection intensity, ruling out the target site mutation as the basis for this phenotype. We instead identified cuticular and metabolic resistance signatures in the resistant colony, and initial findings suggest these resistance mechanisms may be linked to the increased parasite loads. Regardless of whether resistance-conferring traits play a direct role in parasite development, these results imply that selection for insecticide resistance in wild mosquitoes could lead to greater permissiveness for *P. falciparum* infection.

1562

TRANSCRIPTOME ANALYSIS OF *ANOPHELES GAMBIAE* MOSQUITOES ASSOCIATED WITH RESISTANCE SELECTION WITH THREE DIFFERENT GROUPS OF AGRICULTURAL PESTICIDES

Christabelle Gba Sadia[1], France-Paraudie A. Kouadio[1], Marius G. Zoh[2], Behi K. Fodjo[1], Benjamin G. Koudou[1], Jean-Marc Bonneville[2], Stéphane Reynaud[2], Jean-Philippe David[2], Chouaïbou S. Mouhamadou[3]

[1]*Nangui Abrogoua University, Abidjan, Côte D'Ivoire,* [2]*Univ. Grenoble-Alpes, Univ. Savoie Mont Blanc, CNRS, LECA, 38000 Grenoble, Grenoble, France,* [3]*Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC, 27695-7508, USA, North Carolina, NC, United States*

There are several indications that agriculture may be increasing mosquito resistance to insecticides. Using RNA sequencing, we analyzed the transcriptomic profile of three resistant mosquito colonies selected with agrochemicals including insecticides, non-insecticides & a mixture of all compounds. The levels of adult resistance to deltamethrin and bendiocarb were compared between selected and non-selected colonies throughout the selection as well as the frequency of *kdr-w* and *ace1* mutations. After 30 generations, an increase in deltamethrin resistance and *kdr-w* frequency were observed in the three colonies with a significant difference at generation 30 in the insecticide- selected colony compared to the non-selected colony ($P < 0.005$). No changes were detected in the bendiocarb susceptibility among the three colonies & in the *ace1* frequency. RNAseq analysis allowed to identify 10357 transcripts highlighting 30 over-transcribed genes presenting a fold-change ≥ 1.5. Up to 14 genes of P450 family were found upregulated in the insecticides selected colony *versus* 7 genes of the same family in each of the other 2 colonies. The other genes included glutathione-S-transferase & esterase families. In addition, a significant enrichment of transcripts encoding cuticle proteins, transporters & enzymes was observed. Polymorphism analysis revealed 50404 SNPs of which more than 1000 had a frequency difference of more than 30% between the selected and susceptible colonies. In conclusion, this study confirms the involvement of all agricultural pesticide groups in the selection of insecticide resistance in malaria mosquitoes. It also showed that the chemical composition might therefore select mosquito populations with different enzymatic arsenals allowing cross-tolerance to unrelated insecticide compounds. Such indirect effect of global landscape pollution on the ability of mosquito populations to resist insecticides of public health interest deserves further attention since it can deeply impact the kinetics of emergence and the nature of resistance mechanisms selected in malaria vectors with a huge impact on control vector strategies.

1563

MODULATION OF MALARIA VECTOR BLOOD-FEEDING SUCCESS IS ASSOCIATED WITH INSECTICIDE-TREATED BED NETS CONTAINING THE PBO RESISTANCE-REDUCING SYNERGIST IN MALAWI

Jessy Goupeyou-Youmsi[1], Lauren M. Cohee[2], Atusaye Simbeye[1], Chifundo Kadangwe[1], Alfred Matengeni[1], Clarissa Valim[3], Miriam K. Laufer[2], Mark L. Wilson[4], Edward D. Walker[5], Charles Mangani[1], Don P. Mathanga[1], Themba Mzilahowa[1]

[1]*University of Malawi College of Medicine, Blantyre, Malawi,* [2]*University of Maryland School of Medicine, Baltimore, MD, United States,* [3]*Boston University, Boston, MA, United States,* [4]*University of Michigan School of Public Health, Ann Arbor, MI, United States,* [5]*Michigan State University, East Lansing, MI, United States*

Use of long-lasting insecticidal nets (LLINs) has reduced the global malaria burden by impacting vector populations. Prior work suggests that piperonyl butoxide (PBO) impregnated nets work better than pyrethroid-only nets, especially in regions with pyrethroid insecticide resistance. We evaluated whether PBO-treated LLINs affected the blood-feeding success of *Anopheles* vectors in two sites in southern Malawi. The study was undertaken during the rainy season (Dec 2019 - Apr 2020) in 10 clusters of households (HHs) around the health centers of two districts that were part of the 2018 nationwide net distribution campaign: Namanolo in Balaka district that received non-PBO LLINs, and Ntaja in Machinga district that received PBO LLINs. A total of 61 HHs were studied: 22 non-PBO HHs and 39 PBO HHs. Mosquitoes were sampled during each HH weekly or bi-weekly visit, first by pyrethrum spray catch, and then by CDC light trap. Mosquito species and female bloodfed status (dichotomous) were determined by microscopy. Overall, 348 HH visits (range 2-7 visits per HH) were made to the non-PBO (N=126) and PBO (N=222) houses. Of 6,585 *Anopheles* spp., 6,213 (94.4%) were females and all were either *Anopheles funestus* group or *Anopheles gambiae s.l.* Of these, 714 (11.5%) were bloodfed *Anopheles* females, including 264/1,444 (18.3%) *An. funestus* and 450/4,769 (9.4%) *An. gambiae s.l.* In the non-PBO area, more bloodfed *An. funestus* females (24.8%; n=157) were captured compared to the PBO area (13.2%; n=107). Similarly, in the non-PBO area, more *An. gambiae s.l.* females (11.6%; n=298) were found than in the PBO HHs (6.9%; n=152). Therefore, the proportion of bloodfed *An. funestus* was 53% lower, and that of *An. gambiae s.l.* 60% lower, at the PBO HHs compared with the non-PBO HHs. These results are consistent with predicted effect of PBO LLINs on malaria vector populations that show resistance to pyrethroid insecticides in LLINs. Decreasing successful blood feeding by more than half should reduce transmission and lower human prevalence of infection with *P. falciparum*. This study supports the WHO recommendation to use PBO LLINs in areas of pyrethroid resistance such as Malawi.

1564

REDUCED LONG-LASTING INSECTICIDAL NET EFFICACY AND PYRETHROID INSECTICIDE RESISTANCE ARE ASSOCIATED WITH OVER-EXPRESSION OF *CYP6P4, CYP6P3* AND *CYP6Z1*IN POPULATIONS OF *ANOPHELES COLUZZII* FROM CÔTE D'IVOIRE

Anne Meiwald[1], Emma Clark[1], Mojca Kristan[1], Constant Edi[2], Claire L. Jeffries[1], Bethanie Pelloquin[1], Thomas Walker[1], **Louisa Alexandra Messenger**[1]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*Centre Suisse de Recherche Scientifique en Côte d'Ivoire, Abidjan 01, Côte D'Ivoire*

The threat of insecticide resistance across sub-Saharan Africa is anticipated to have severe implications for the continued effectiveness of malaria vector control interventions. First reports of pyrethroid and carbamate resistance in Côte d'Ivoire date back to the early 1990s. Nowadays, resistance to major public health insecticides is pervasive, driven by contemporaneous scale-up of malaria control measures and use of agricultural pesticides. This study evaluated the bioefficacy of conventional and next-generation long-lasting insecticidal nets (LLINs), against wild *Anopheles coluzzii*; determined current insecticide resistance profiles using CDC intensity bioassays, including assessing the toxicity of candidate insecticides, chlorfenapyr and clothianidin; and characterised underlying target site and metabolic resistance mechanisms, to safeguard future malaria control efforts in Côte d'Ivoire. Pyrethroid resistance was intense, with more than 25% of mosquitoes surviving exposure to ten times the doses of alpha-cypermethrin, deltamethrin and permethrin required to kill a susceptible population. Similarly, levels of knock-down (KD) and 24-hour mortality to LLINs containing only deltamethrin were very low (0.56% and 5.44%, respectively) and not significantly different to an untreated net (1.56% and 6.11%, respectively). By comparison, LLINs containing deltamethrin and the synergist piperonyl butoxide (PBO) performed significantly better (KD and 24-hour mortality of 79.78% and 83.81%, respectively). Field populations were also susceptible to clothianidin (mean 72-hour mortality of 94.11%) and chlorfenapyr (95.54%). Pyrethroid resistance was associated with high frequencies of the L1014F *kdr* mutation (87.8%) and significant over-expression of *CYP6P4* (Fold Change=5.88), *CPY6Z1* (FC=4.04) and *CYP6P3* (FC=12.56). Study findings raise concerns regarding the potential operational failure of standard

LLINs and support the deployment of next-generation vector control interventions incorporating PBO, chlorfenapyr and clothianidin, in areas of high resistance intensity in Côte d'Ivoire.

1565

THE ROLE OF VAGINAL INFLAMMATION IN HIV VULNERABILITY IN ZAMBIAN WOMEN WITH FEMALE GENITAL SCHISTOSOMIASIS

Amy S. Sturt[1], Emily L. Webb[1], Catriona Patterson[1], Comfort R. Phiri[2], Bellington Vwalika[3], Eyrun F. Kjetland[4], Maina Mudena[5], Joyce Mapani[5], Mabel Mutengo[6], James Chipeta[3], Govert J. van Dam[7], Paul L. Corstjens[7], Helen Ayles[2], Richard J. Hayes[1], Grace McComsey[8], Isaiah Hansingo[5], Piet Cools[9], Lisette A. van Lieshout[7], Suzanna C. Francis[1], Amaya L. Bustinduy[1]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*Zambart, Lusaka, Zambia,* [3]*University of Zambia School of Medicine, Lusaka, Zambia,* [4]*Oslo University Hospital, Oslo, Norway,* [5]*Livingstone Central Hospital, Livingstone, Zambia,* [6]*Levy Mwanawasa Medical University, Lusaka, Zambia,* [7]*Leiden University Medical Center, Leiden, Netherlands,* [8]*Case Western Reserve University, Cleveland, OH, United States,* [9]*Ghent University, Ghent, Belgium*

HIV infection disproportionately affects women in sub-Saharan Africa, where areas of high HIV prevalence and *Schistosoma haematobium* endemicity overlap. Female Genital Schistosomiasis (FGS), caused by *S. haematobium* egg deposition in the genital tract, has been associated with prevalent HIV infection. We hypothesize that FGS-related vaginal inflammation may provide a causal mechanism for the association between FGS and HIV. Elevated levels of inflammatory cytokines MIP-1**α**;, MIP-1**β**;, IP-10, and IL-8 have been associated with HIV acquisition. In this cross-sectional study, we evaluated vaginal inflammatory cytokines in women with and without FGS. Specimens were collected from 603 female participants who were aged 18-31 years, sexually active, and not pregnant from the HPTN 071 (PopART) HIV prevention trial in Zambia. Participants self-collected urine, vaginal, and cervical swabs, and a clinician obtained cervicovaginal lavage (CVL). Both microscopy and *Schistosoma* circulating anodic antigen (CAA) testing were performed on urine specimens. Genital samples (CVL, cervical and vaginal swabs) were examined for parasite-specific DNA by PCR. Women with FGS (n=28), defined as a positive genital PCR, were frequency matched by age with 159 controls (all *Schistosoma* diagnostic tests negative). The concentrations of 17 soluble immune proteins (IL1 **α**;, IL-1 **β**;, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-15, IL-17A, IP-10, MCP-1, MIP-1**α**;, MIP-1**β**;, IFN-γ, TNF-**α**; and eotaxin) were quantified by multiplex bead-based immunoassays. There was no difference in concentrations of inflammatory biomarkers between participants with and without FGS. An ad hoc subgroup analysis including women with ≥2 genital specimens positive for *Schistosoma* DNA by PCR (n=15) showed that women with FGS had higher levels of IL-4, IL-5, IL-15, and IL-13 compared to women without FGS ($p<0.05$). After adjustment for multiple comparisons, the association was unlikely to have occurred by chance ($p<0.02$). FGS may alter the female genital tract immune environment, but larger studies in areas of varying endemicity are needed to evaluate the association with HIV vulnerability.

1566

ENHANCING CATHEPSIN B RESPONSES OF YS1646 *SALMONELLA* TYPHIMURIUM VECTORED VACCINATION USING MUCOSAL ADJUVANTS IN A MURINE SCHISTOSOMIASIS MODEL

Adam S. Hassan, Dilhan J. Perera, Brian J. Ward, Momar Ndao

Research Institute of the McGill University Health Centre, Montreal, QC, Canada

Caused by trematode worms of the genus *Schistosoma*, schistosomiasis is considered the most important human helminth disease by the WHO. *S. mansoni* juveniles traverse the lung mucosa and adults reside adjacent to the gut mucosa. Several candidate vaccines are in pre-clinical and clinical development, but none is designed to elicit a mucosal response. We have exploited an attenuated *Salmonella enterica* Typhimurium strain (YS1646) to produce a vaccine that targets Cathepsin B (CatB), a digestive enzyme important for both juveniles and adult worms. Our previous studies based on this vector resulted in 80-90% reduction in parasite burden following a 21-day prime-boost regimen in our C57BL/6 mouse challenge model. Although promising, a more convenient immunization schedule would likely increase vaccine compliance in endemic areas. Herein we present data for a 5-day, multi-modality schedule consisting of simultaneous oral (PO) and intramuscular (IM) vaccination on day 1 (D1) followed by two additional PO doses on D3 and D5. The four groups studied were i) saline control, ii) the 'empty' YS1646 vector with IM recombinant CatB (rCatB), iii) PO YS1646-CatB alone, iv) PO YS1646-CatB with IM rCatB (PO+IM). Animals were challenged 3 weeks post-vaccination with 150 cercariae by tail penetration. Worm numbers and intestinal/liver egg burden were reduced 82.1% and 83.2%/73.8% in the PO+IM group compared to the saline control group, respectively. We also investigated the potential benefits of two mucosal adjuvants in this short vaccination schedule: all-trans retinoic acid (ATRA) and double-mutant heat-labile toxin (dmLT). CatB-specific serum IgG responses measured by ELISA were increased from 186.3 ng/mL in the unadjuvanted PO+IM group to 482.5 ng/mL and 772.4 ng/mL when adjuvanted by 10μg ATRA or 10μg dmLT respectively. Similarly, intestinal CatB-specific IgA responses rose from 458.4 ng/g of intestine without adjuvant to 823.5 ng/g or 882.2 ng/g when adjuvanted by ATRA and dmLT respectively. The impact of these adjuvants on cellular immune responses as well as challenge will be assessed in future studies as soon as COVID-19 restrictions are lifted.

1567

H06-IPSE, A PATHOGEN-SECRETED HOST NUCLEUS-INFILTRATING PROTEIN (INFILTRIN), VARIES IN INTERNALIZATION MECHANISM AND EFFICIENCY BY TARGET CELL TYPE

Olivia K. Lamanna[1], Evaristus Mbanefo[1], Kenji Ishida[1], Franco Falcone[2], Theodore Jardetzky[3], Luke Pennington[3], Michael Hsieh[1]

[1]*Children's National Medical Center, Washington, DC, United States,* [2]*University of Giessen, Gießen, Germany,* [3]*Stanford University, Stanford, CA, United States*

IPSE (IL-4 Inducing Principle from *Schistosoma mansoni* Eggs) is the most abundant egg secreted protein of the *Schistosoma* parasite. As an immunomodulatory protein, IPSE binds IgE on basophils to induce IL-4 secretion, triggers Breg cell activation, and, as an infiltrin, translocates into host cell nuclei to alter transcription. Given these functions, we are developing IPSE as a therapeutic for inflammatory disorders. We reported that H03 (one of two major *Schistosoma haematobium* orthologs of IPSE, the other being H06) has varying internalization efficiencies among urothelial (most efficient), hepatocyte, immature dendritic, endothelial, and neuronal (least efficient) cells. Also, urothelial cells mainly internalize H03 through clathrin-mediated endocytosis (CME). Prior data indicate H06 decreases capsaicin receptor-mediated bladder pain. Our objective was to characterize H06 internalization by urothelial and neuronal cells (both important in bladder pain). Urothelial and neuronal cells were incubated with a CME inhibitor and then Alexa-488 conjugated H06 or Alexa-488-conjugated transferrin (CME internalization control). Flow cytometry with extracellular signal quenching was used to quantify internalization. The CME inhibitor reduced H06 internalization by 89% in urothelial cells, compared to 40% in neurons. Decreased transferrin uptake by neurons versus urothelial cells suggests neurons feature less CME. Despite this variation, H06 internalization by urothelial and neuronal cells was uniform (mean ~30%). This data suggest H06 was selectively internalized by neurons, consistent with our hypothesis that H06 acts through the capsaicin receptor on nociceptive neurons to alleviate capsaicin receptor-mediated pain. H06, similar to other capsaicin receptor agonists, may bind to this receptor and undergoes CME, thus removing the receptor from the membrane, rendering it unavailable for further binding. Ongoing research

is examining H06's interaction with the capsaicin receptor, endosomal escape mechanisms, and nuclear activity to understand its role in the pathogenesis of schistosomiasis and as an analgesic.

1570

A PILOT INVESTIGATION OF SCHISTOSOME HYBRIDS: A MORPHOLOGICAL CHARACTERIZATION OF *SCHISTOSOMA HAEMATOBIUM* EGGS, WITHIN A PUTATIVE HYBRID ZONE IN OGUN STATE, NIGERIA

Uwemedimo Friday Ekpo[1], Adedotun Ayodeji Bayegun[1], Foluke Adebayo Akande[1], Kehinde Olutoyin Ademolu[1], Russell John Stothard[2]

[1]*Federal University of Agriculture Abeokuta, Abeokuta, Nigeria,* [2]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom*

Urogenital schistosomiasis is a waterborne disease of global concern, with a newly emerging epidemiological dynamic in sub-Saharan Africa. There are now growing concerns of pervasive hybridization within the *Schistosoma haematobium* group; hybrids suspected to have expanded transmission potential or altered virulence/drug tolerance. In line with recent World Health Organisation recommendations, we expand our national surveillance of *S. haematobium* in Nigeria for a pilot study of schistosome hybrids. A parasitological screening of urines samples from 99 community members and faecal samples from 70 cattle in Apojula, Ogun State, Nigeria were performed. Apojula is a potential hybrid zone between the human *S. haematobium* and the bovine *S. bovis*. The presence of atypical eggs with unusual shapes and morphology was noted and recorded. Eggs were photographed and analyzed with IC Measure™ (The Imaging Source, USA) measuring the total length and maximum width (µm) and a ratio of egg shape (length divided by width). Data were tabulated using Microsoft Excel 2016 with analysis by IBM SPSS 20.0 software. Prevalence of egg-patent infection in human was 56.6%. A total of 7,273 eggs recovered of which 251 eggs were photographed and analyzed; 241 eggs possessed terminal spines while 10 eggs were spineless. Egg-shaped showed slight variations as the majority (233 eggs, 92.9%) were round-to-oval in line with typical *S. haematobium* eggs, while (18 eggs, 7.1%) were spindle-shaped. Total egg length ranged from 79.8 - 244.2µm and mean of 184.2µm. Maximum width ranged from 33.4 - 105.7µm and mean of 81.47µm. The length/width ratio ranged from 1.3 - 3.4µm and mean of 2.3µm with scatter plots exhibiting an uneven distribution. Only one cattle was infected with *S. bovis* accounting for a prevalence of 1.43%. Analysis of our initial findings strongly suggests the possibility of hybrid species occurring here. As egg morphology is known to be an insensitive indicator of detecting hybridization, we will augment our analysis within an inspection of genetic loci of schistosome eggs stored in ethanol.

1571

MAPPING *SCHISTOSOMA HAEMATOBIUM* FOR NOVEL INTERVENTIONS AGAINST FEMALE GENITAL SCHISTOSOMIASIS AND ASSOCIATED HIV RISK IN KWAZULU-NATAL, SOUTH AFRICA

Mahala Livingston

Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States

Schistosoma haematobium is a waterborne parasitic infection endemic to Sub-Saharan Africa associated with urogenital disease. In addition to their debilitating genitourinary symptoms, women with Female Genital Schistosomiasis (FGS) have been found to have a 3-fold higher risk of HIV infection. Despite WHO recommendations, few schools in schistosomiasis and HIV co-endemic areas of South Africa receive regular antischistosomal mass drug administration (MDA) due to a lack of updated epidemiological data. To provide data for future preventative efforts against FGS and HIV this study explored S. haematobium prevalence in girls and young women and the effects of antischistosomal MDA. Urinary schistosomiasis and genital symptom prevalence were investigated in 70 randomly selected secondary schools in 3 districts in KwaZulu-Natal and 18 primary schools. All study participants were treated for schistosomiasis and schools with high urinary prevalence were followed up after 1 and 4 years of MDA. Prevalence and potentially infested rivers were mapped across these districts based on lab and survey data. At baseline, most schools fell within the WHO "medium risk" prevalence category necessitating biennial antischistosomal MDA per WHO guidelines. Interestingly, young women demonstrated high rates of genital symptoms even after correcting for sexually transmitted infections. These symptoms may be explained by S. haematobium infection undiagnosed by urine analysis alone.

1572

WATER, SANITATION, AND ANIMAL-ASSOCIATED RISK FACTORS FOR ENTERIC PATHOGEN EXPOSURE IN THE VACCINE IMPACT ON DIARRHEA IN AFRICA (VIDA) STUDY: THE GAMBIA, KENYA, AND MALI, 2015-2018

David Berendes[1], Graeme Prentice-Mott[1], Kirsten Fagerli[1], Sunkyung Kim[1], Dilruba Nasrin[2], Helen Powell[3], Irene Kasumba[2], Sharon Tennant[2], Anna Roose[2], M. Jahangir Hossain[4], Joquina Chiquita M. Jones[4], Syed M. Zaman[4], Richard Omore[5], John Benjamin Ochieng[5], Jennifer Verani[6], Marc-Alain Widdowson[6], Samba Sow[2], Dramane Malle[7], Sanogo Doh[7], Ciara O'Reilly[1], Jie Liu[8], James Platts-Mills[8], Eric Houpt[8], Karen Kotloff[2], Eric Mintz[1]

[1]*Division of Foodborne, Waterborne, and Environmental Diseases, Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Department of Medicine, Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States,* [3]*Department of Pediatrics, Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, United States,* [4]*Medical Research Council Unit The Gambia at the London School of Hygiene & Tropical Medicine, Banjul, Gambia,* [5]*Center for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya,* [6]*Division of Global Health Protection, Centers for Disease Control and Prevention, Nairobi, Kenya,* [7]*Center for Vaccine Development-Mali, Bamako, Mali,* [8]*University of Virginia, Charlottesville, VA, United States*

Recent evidence suggests higher pediatric exposure to enteric pathogens in low-income settings than previously recognized, requiring more substantive water and sanitation interventions, including animal feces management. To investigate pathogen-specific environmental risks, this study assessed enteric pathogen exposure measures by water, sanitation, and animal-associated risk factors in the Vaccine Impact on Diarrhea in Africa case-control study of <5 year-olds in The Gambia, Kenya, and Mali. We assessed enteric pathogens detected in stool via TaqMan Array Card in cases of moderate-to-severe diarrhea and their controls and surveyed caregivers about household water and sanitation conditions (using WHO/UNICEF service ladder criteria) and animals living in the compound. Risk ratios (RRs) and 95% confidence intervals (CIs) were calculated using modified Poisson regression adjusted for age, sex, caregiver's education, household assets, and fuel source, with separate models for cases (CA) to controls (CO). In stools of the 11,053 children enrolled, enteric bacteria (81% of children), viruses (53%), and protozoa (35%) were prevalent, most commonly enteroaggregative *E. coli* (62%), *Campylobacter* spp. (48%), and *Shigella* spp. (28%). For both CA and CO, fowl and goats living in the compound were associated with *Campylobacter* spp. (fowl: RR_{CA}: 1.11 [95% CI: 1.03, 1.20], RR_{CO}: 1.15 [1.07, 1.24]; goats: 1.10 [1.00, 1.20], 1.09 [1.00, 1.19]). In CA but not CO, unimproved sanitation, and cows and sheep living in the compound were associated with Shiga toxin-producing *E. coli* (STEC) (unimproved sanitation: 1.56 [1.12, 2.17]; cows: 1.61 [1.16, 2.24]; sheep: 1.48 [1.11, 1.96]). In CO but not CA: surface water was associated with *Cryptosporidium* spp. (1.45 [1.06, 1.98]), heat-stable toxin-producing enterotoxigenic *E. coli* (ST-ETEC) (1.26 [1.00, 1.60]), and *Shigella* spp. (1.34 [1.04, 1.72]); and access to basic water sources with *Shigella* spp. (1.44 [1.12, 1.86]). Findings underscore pathogen exposure risks from drinking surface water and poor sanitation, but also potentially from animals living in low-income settings.

1573

CAMPYLOBACTER INFECTION AND HOUSEHOLD FACTORS ARE ASSOCIATED WITH CHILDHOOD GROWTH IN URBAN BANGLADESH

J. Johanna Sanchez[1], Md. Ashraful Alam[2], Christopher B. Stride[3], Md. Ahshanul Haque[2], Subhasish Das[2], Mustafa Mahfuz[2], Daniel E. Roth[4], Peter D. Sly[5], Kurt Z. Long[6], Tahmeed Ahmed[2]

[1]*University of Queensland/University of Toronto, Toronto, ON, Canada,* [2]*icddr,b, Dhaka, Bangladesh,* [3]*University of Sheffield, Sheffield, United Kingdom,* [4]*Hospital for Sick Children and University of Toronto, Toronto, ON, Canada,* [5]*University of Queensland, Brisbane, Australia,* [6]*Swiss Tropical and Public Health Institute, Basel, Switzerland*

Enteric infection and childhood malnutrition continues to be a global health concern and a leading cause of morbidity and death among children. *Campylobacter* infection, in particular, is highly prevalent in low- and middle-income countries, including Bangladesh. Using longitudinal data from 265 children participating in the Bangladesh MAL-ED Study site, we applied latent growth curve modelling (LGCM) to evaluate the trajectories of change in children's height, as measured by length-for-age z-score (LAZ), from age 0-24 months. We also identified associations with infection and household factors. LGCM is a form of longitudinal analysis that utilizes a structural equation modelling framework to model change in an outcome. *Campylobacter* infections, both symptomatic and asymptomatic, were included as 3- and 6-month lagged time-varying covariates, while household risk factors were included as time-invariant covariates. In the final model, maternal height and child birth order were positively associated with LAZ at birth. *Campylobacter* infection in the preceding 3-month interval was negatively associated with LAZ at 12, 15, and 18 months of age. Similarly, infection in the preceding 6-month interval was negatively associated with LAZ at 15, 18, and 21 months of age. Duration of antibiotic use and treated drinking water were negatively associated with infection, with the strength of the effect of treated drinking water increasing with age. *Campylobacter* infection had a significant negative effect on child's growth and this was most powerful between 12 and 21 months. Access to treated drinking water and increased antibiotic use had a positive indirect effect on linear child growth trajectory, acting via their association with *Campylobacter* infection. To our knowledge, we describe the first application of LGCM to explore *Campylobacter* infection and linear growth in children. This method is less commonly used outside of the social sciences and provides a novel approach for examining enteric infection.

1574

COMPARING GUT BACTERIAL MICROBIOMES AND ANTIMICROBIAL RESISTOMES BETWEEN HUMANS, CHICKENS, AND GOATS IN URBAN AND RURAL BANGLADESH

Jenna Swarthout[1], Erica R. Fuhrmeister[1], Latifah Hamzah[2], Angela R. Harris[3], Emily S. Gurley[4], Syed M. Satter[5], Alexandria B. Boehm[2], Amy J. Pickering[1]

[1]*Tufts University, Medford, MA, United States,* [2]*Stanford University, Stanford, CA, United States,* [3]*North Carolina State University, Raleigh, NC, United States,* [4]*Johns Hopkins University, Baltimore, MD, United States,* [5]*International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh*

Urban populations are increasing in low- and middle-income countries (LMICs), which will likely bear the largest burden of mortality due to antimicrobial-resistant (AMR) infections. Due to insufficient sanitation in LMICs, AMR bacteria, selected through antibiotic misuse in humans and animals, disseminate into the environment. Exchange of AMR bacteria and antimicrobial resistance genes (ARGs) between humans, animals, and the environment has been demonstrated. Urban settings could be hotspots of zoonotic antimicrobial resistance transmission due to inadequate water, sanitation, and hygiene infrastructure and cohabitation of humans and animals. Our primary objective was to assess whether human and animal gut microbiomes and resistomes were more similar in urban compared to rural Bangladesh using amplicon and long-read (nanopore) sequencing. 120 total human, goat, and chicken fecal samples were collected from one urban and one rural community in Bangladesh to create four fecal composites for each host within each community. Gut microbiomes and resistomes were not more similar between humans and animals in urban versus rural Bangladesh (Bray-Curtis dissimilarity, Wilcoxon rank-sum test, $p>0.05$ for all tests). The pooled abundance of ARGs (normalized by Gbp of data classified as bacteria) was greater across all fecal hosts in urban versus rural communities. Within human and goat samples, gut bacterial communities differed between urban and rural samples from the respective host (PERMANOVA, $p=0.001$). The percentage of shared unique ARGs between humans and goats was greater (Z-test, $p=0.007$), while unique ARG sharing between humans and chickens was not different (Z-test, $p>0.05$), in urban versus rural Bangladesh. Using long reads, we identified potentially pathogenic bacterial species carrying ARGs and will also present our results in the context of plasmid-mediated resistance. The high abundance and sharing of ARGs in human and animal hosts in urban Bangladesh highlight it as a hotspot of AMR transmission. Environmental interventions to tackle AMR in urban and rural settings should account for both human and animal reservoirs.

1575

PATHOGEN FLOWS FROM ON-SITE SANITATION SYSTEMS IN LOW-INCOME URBAN NEIGHBORHOODS, DHAKA: A QUANTITATIVE ENVIRONMENTAL ASSESSMENT

Md. Nuhu Amin[1], Pengbo Liu[2], Tim Foster[3], Mahbubur Rahman[1], Md. Rana Miah[1], Golam Bashir Ahmed[1], Mamun Kabir[1], Suraja Raj[2], Guy Norman[4], Christine L. Moe[2], Juliet Willetts[3]

[1]*Infectious Diseases Division, International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh,* [2]*Center for Global Safe Water, Sanitation, and Hygiene, Emory University, Atlanta, GA, United States,* [3]*Institute for Sustainable Futures, University of Technology Sydney, Sydney, Australia,* [4]*Water and Sanitation for the Urban Poor (WSUP), London, United Kingdom*

Despite wide usage of on-site sanitation, there is limited field-based evidence on the removal or release of pathogens from septic tanks and other primary treatment systems such as anaerobic baffled reactors (ABR). In two low-income areas in Dhaka, we conducted a cross-sectional study to explore pathogen loads discharged from commonly used on-site sanitation-systems and their transport to nearby drains and waterways. We collected samples of drain water, drain sediment, canal water and floodwater from April-October 2019. Sludge, supernatant, and effluent samples were also collected from septic tanks and ABRs. We investigated the presence and concentration of selected enteric pathogen (*Shigella*/EIEC, *V.cholerae*, *S.* Typhi*,* Norovirus Genogroup-II (NoV-GII)*,* and *Giardia*) and presence of *Cryptosporidium* in these samples using quantitative polymerase chain reaction (qPCR). Among all samples tested (N=150), 89% were contaminated with *Shigella*/EIEC, 69% with *V. cholerae*, 65% with NoV-GII, 32% with Giardia, 17% with *S.* Typhi and 6% with *cryptosporidium*. A high concentration of pathogens [range: mean $\log_{10}$=1.41 Equivalent genome copies (EGC)/100mL in canal grab samples to 7.04 EGC/100 mL in ABR sludge] was found in most samples. In particular, high concentrations of pathogens were detected in septic tank effluent [range: mean $\log_{10}$ concentration=3.07-4.34 EGC/100 mL] and ABR effluent [range: mean $\log_{10}$ concentration=2.57-4.94 EGC/100 mL]. High concentrations of pathogens (particularly NoV-GII, *V.cholerae* and *Shigella*/EIEC) were frequently detected in environmental samples from the two study areas and at high concentrations. The numerous environmental exposure pathways for children and adults make these findings of public health concern. These alarming results should prompt rethinking of how to achieve safe sanitation solutions that protect public health in dense low-income areas. Future studies should assess changes in pathogen exposure associated with specific sanitation interventions and consider the health impacts of environmental contamination among both high-income and low-income communities in Dhaka city.

1576

ENVIRONMENTAL TRANSMISSION OF DRUG RESISTANT BACTERIA IN COMMUNITY THROUGH WASTE WATER RUN-OFF IN BANGLADESH- PLANETARY HEALTH EVIDENCE OF ANTIBIOTIC RESISTANCE

Muhammad Asaduzzaman[1], Emily Rousham[2], Mohammad Aminul Islam[3]

[1]*Centre for Global Health, Institute of Health and Society, University of Oslo, Oslo, Norway,* [2]*Centre for Global Health and Human Development, School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, United Kingdom,* [3]*Laboratory Sciences and Services Division, icddr,b, Dhaka, Bangladesh*

Antimicrobial resistance (AMR) is a global health threat with increasing evidence of resistant bacteria in the environment. The aim of our study was to investigate resistant bacterial loads in wastewater runoff from the point of disposal to the downstream surface water bodies in Bangladesh. In 2018, we conducted two surveys (winter and summer) of wastewater and surface water samples in three settings: urban live bird markets, small-scale poultry farms and rural households. Wastewater samples were collected from the primary disposal point of each farm, household or market. Surface water samples included downstream pond and river water. All samples were analyzed to estimate the prevalence of *extended spectrum beta lactamase-producing E. coli (ESBL-Ec)* and *carbapenem resistant E. coli (CREc)* per ml of water sample. *ESBL-Ec* and *CREc* were present in water samples from all settings. The prevalence of ESBL-Ec in wastewater samples was 90%, 90% and 83% in rural households (n=40), farms (n=40) and urban markets (n=40) respectively, whereas the corresponding CREc prevalence was 8%, 5% and 8% . River water samples had a higher prevalence of ESBL-Ec (85%) and CREc (22%) compared to pond water (71% and 7% respectively). Therefore, *E. coli* counts were higher in waste water samples compared to pond and river water. Of all wastewater samples, mean count of both *ESBL-Ec* (log10 5.26 ± 1.32) and *CREc* ($\log_{10}$ 3.05 ± 0.25) were highest in urban markets compared to farm and rural samples. In all three settings, we found no statistically significant seasonal difference in *ESBL* prevalence except the river water in poultry farm (p=0.041) where the prevalence was more in winter. The highest prevalence and concentration of third-generation cephalosporin and carbapenem resistant *E. coli* in wastewater samples across all study sites, renders human and animal species at risk of exposure to these organisms.These findings emphasize to focus on AMR as a planetary health agenda and an ecosystem approach including environmental «hotspot» identification and nature based solutions needs to be applied to combat this multidisciplinary issue.

1577

IMPACT OF WASH CONDITIONS ON MICROBIAL CONTAMINATION OF THE ENVIRONMENT IN TWO HOSPITALS IN AMHARA, ETHIOPIA

Kristen Carr[1], Kun Zhao[2], Habib Yakubu[1], Lamesgin Alamineh[3], Mulusew Belew[3], Abebe Gebremariam[4], Gizachew Yismaw[5], John Cranmer[4], Christine L. Moe[1]

[1]*Center for Global Safe Water, Sanitation, and Health at Emory University, Atlanta, GA, United States,* [2]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [3]*Emory Ethiopia - Amhara Regional Office, Bahir Dar, Ethiopia,* [4]*Emory University-Nell Hodgson Woodruff School of Nursing, Atlanta, GA, United States,* [5]*Amhara Public Health Institute, Bahir Dar, Ethiopia*

Water, sanitation, and hygiene (WASH) infrastructure and practices in health care facilities (HCF) may impact risk of healthcare-associated infections, such as sepsis, in infants. Despite infection prevention benefits, WASH conditions in HCFs are poor in many global contexts, and there is little evidence on the impact of WASH conditions on HCF contamination and risks of neonatal sepsis. This study examined the relationship between WASH conditions and environmental contamination in maternity wards and neonatal intensive care units in two large Ethiopian hospitals with high incidence of neonatal sepsis. WASH conditions were systematically assessed every 2-4 weeks using the WASHCon tool over 32 weeks, and environmental contamination was measured concurrently by collection of surface swabs, handrinses, drinking and device water samples. WASHCon assessed hand hygiene, infection prevention, environmental cleanliness, sanitation, water availability and quality. Composite scores were calculated by hospital, ward, and time. Over 440 samples were analyzed for *S. aureus*, *E. coli*, and total coliforms and were matched to WASHCon scores by hospital, ward, and time. Both hospitals had challenges with water availability, water and soap for hand hygiene, and safe segregation of waste. Problems with visible cleanliness were noted in the rural hospital which had fewer clinical and support staff. In this hospital, *E. coli* (fecal indicator and frequent cause of sepsis) was detected in 23% of surface swabs, 21% of handrinses, and 16% of drinking water samples. In the urban hospital, *E. coli* was not detected in drinking water, but 5% of swabs and 10% of handrinses were positive for *E. coli*. In the rural hospital, the highest *E. coli* detection rates were in the Kangaroo Mother Care (KMC) ward (37% of samples), followed by the post-natal ward (20%)– both wards where mothers and neonates are together. In the urban hospital, overall *E. coli* detection rates were lower and ranged from 4% in the KMC ward to 6% in the post-natal ward. Optimizing water and soap availability and environmental cleaning could decrease infant exposure to sepsis pathogens in HCFs.

1578

HOME WASH CONDITIONS AND POST-CESAREAN SECTION SURGICAL SITE INFECTION RISKS

Sadoscar Hazikimana[1], **Ildephonse Simbananiye**[2], Elizabeth Miranda[3], Anne Niyigena[1], Laban Bikorimana[1], Andrea Goodman[4], Theoneste Nkurunziza[5], Robert Riviello[6], Bethany Hedt-Gauthier[4], Fredrick Kateera[1]

[1]*Partners in Health - Rwanda, Kigali, Rwanda,* [2]*Ministry of Health, Kigali, Rwanda,* [3]*Program in Global Surgery and Social Change, Harvard Medical School, Boston, MA, United States,* [4]*Department of Global Health and Social Medicine, Harvard Medical School, Boston, MA, United States,* [5]*Technical University of Munich, Munich, Germany,* [6]*Center for Surgery and Public Health, Brigham and Women's Hospital, Boston, MA, United States*

Adequate water, sanitation and hygiene (WASH) conditions are crucial to health and wellbeing. Surgical site infections (SSIs) are the most common post-cesarean section (c-section) complication in Africa, and our previous work has linked SSIs to inadequate WASH conditions at health facilities. However, to our knowledge, no research has studied the associations of WASH conditions in patients' homes with post-c-section SSI in rural Africa. In this study, we enrolled all mothers who underwent c-section at Kirehe District Hospital in rural Rwanda, between September 2019 and February 2020. Study trained data officers recorded demographics on postoperative days (PODs) 1 and 3 via clinical chart review and patient interviews. Patient home WASH conditions were assessed by community health workers (CHWs) on POD10. Patients returned to the hospital at POD11 for general practitioner assessment and diagnosis of SSI. We assessed the relationship between home WASH conditions and SSI using Fisher's exact test. 808 women were enrolled, and 710 (87.9%) completed both the home and the POD11 follow-up visits. Regarding the primary water source used for baths at home, most (49%, n=351) reported using piped water collected from locations outside of their homes, and 13.3% (n=95) reported using a protected spring. Notably, 5% (n=37) had not bathed since discharge due to lack of water. A third (n=223, 31.4%) of women did not regularly treat their water, and 149 (21%) had no hand washing station at home. At POD11, 38 women (5.3%) were diagnosed with SSI. Inability to bathe because of lack of water (p=0.011) was significantly associated with SSI, while lack of regular treatment of drinking water was borderline (p=0.046). No significant association was seen between SSI and other WASH variables. In conclusion, improved WASH conditions play a large role in prevention of post-c-section SSI, and, in our study, a significant

association between SSI and inability to bathe since discharge was found. More research should be done to see how home WASH conditions can be improved in order to improve patients outcomes after surgery.

1579

ECHOCARDIOGRAPHIC PREDICTORS OF MORTALITY IN PATIENTS WITH CHAGAS DISEASE FROM REMOTE ENDEMIC AREAS: SAMI-TROP COHORT STUDY

Maria Do Carmo P. Nunes[1], Marcelo Maia[2], Antonio Ribeiro[1], Claudia Oliveira[3], Ariela Ferreira[2], Lea Campos Oliveira[4], Ana Luiza Bierrenbach[5], Desireé Haikal[2], Larissa Martins[1], Clareci Cardoso[3], Ester Sabino[6]

[1]*Universidade Federal de Minas Gerais, Belo Horizonte, Brazil,* [2]*Universidade Estadual de Montes Claros, Montes Claros, Brazil,* [3]*Federal University of São João del-Rei, Divinópolis, Brazil,* [4]*Hospital das Clinicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil,* [5]*Hospital Sírio-Libanês, São Paulo, Brazil,* [6]*Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil*

Patients with Chagas disease are at increased risk of premature death. Echocardiography provides accurately assessment of ventricular function, which is essential for clinical decision making. We investigated conventional echocardiographic parameters that may predict mortality in patients Chagas disease who live in remote poor areas with limited-resource setting. A total of 370 patients from a large cohort (SaMi-Trop) of Chagas disease living in the north of Minas Gerais State, Brazil, who presented with abnormal electrocardiogram were prospectively enrolled. The endpoint was all-cause mortality. A range of readily obtained echocardiographic measures were collected using portable equipment. The mean age was 66 ± 13 years and 207 (56%) were women. The majority of the patients had left ventricular (LV) systolic dysfunction with mean ejection fraction of 41 ± 12%. During a mean follow-up of 11 months, 138 patients died (37%). Three key echocardiographic parameters were predictors of mortality in the final model, including LV ejection fraction (HR: 0.95, 95% CI: 0.93 to 0.97; p<0.001), right ventricular dimension (HR: 1.08, 95% CI: 1.03 to 1.12; p<0.001), and E/e' ratio (HR: 1.04, 95% CI: 1.01 to 1.08; p=0.019), after adjustment for age (HR: 1.03, 95% CI: 1.01 to 1.05; p=0.003). The discrimination of the model was good with C-statistic of 0.74, 95% CI 0.69 to 0.79). In a cohort of patients with Chagas disease from endemic area, LV systolic function, diastolic function expressed by E/e' ratio, and right ventricular dimension are associated with mortality and may be the most relevant echocardiographic markers for prognosis in this population.

1580

NAT2 GENETIC VARIATIONS AMONG CHILDREN INFECTED WITH *PLASMODIUM FALCIPARUM* MALARIA IN YAOUNDE, CAMEROON: IMPLICATIONS FOR ANTI-MALARIAL DRUG DOSING, METABOLISM AND RESPONSE

Peter Thelma Ngwa P. Niba[1], Akindeh Mbuh A. Nji[1], Innocent Mbulli I. Ali[2], Lawrence Fonyonga L. Akam[1], Cedric Hermann C. Dongmo[1], Calvino Tah C. Fomboh[1], Jean Paul J. Chedjou[1], Selly Ngaloumo Abdel S. Aziz[1], Jude D. J. Bigoga[1], Michael M. Alifrangis[3], Wilfred Fon W. Mbacham[1]

[1]*University of Yaounde I, Yaounde, Cameroon,* [2]*University of Dschang, Dschang, Cameroon,* [3]*University of Copenhagen, Copenhagen, Denmark*

The arylamine N-acetyltransferase 2 (NAT2) is one of the key phase II liver enzymes actively involved in the metabolism of anti-malarial drugs, antibiotics, and xenobiotics. The presence of point mutations is a well-established phenomenon in the NAT2 gene. The Genetic variation is responsible for the change in N-acetylation activity and these differences N-acetylation activity leads to classification of the population into various phenotypes such as slow, intermediate and rapid acetylators. Individuals with slow drug metabolic status are more likely to be associated with adverse drug events due to toxicity. This eventually leads to treatment failure. The present study aimed to determine NAT2 genetic variations among children infected with *Plasmodium falciparum* malaria in Yaounde, Cameroon as well as determine its role in anti-malarial drug dosing, metabolism and response. Human DNA was extracted using the EZNA Biotek DNA extraction kit from 121 D0 whole blood samples collected during a randomized controlled clinical trial on artesunate-amodiaquine and artemether-lumefantrine from May 2019 to April 2020 in Yaounde, Cameroon. The extracted DNA samples were genotyped by Polymerase Chain Reaction (PCR) and digested with restriction enzymes KpnI for (NAT2*5, C481T), Taq$^{\alpha}$I for (NAT2*6, G590A) and BamHI for (NAT2*7, G857A). The genetic variants with the highest frequencies were NAT2*5/7 (46.3%), NAT2*4/7 (26.4%), and NAT2*6/7 (19.0%). The slow acetylator phenotypes defined by NAT2*5/5, NAT2*5/7, NAT2*6/7, and NAT2*7/7 genotypes were the predominance with 69.4% while those with intermediate had 26.4%. There was a significant association between gene polymorphisms (C481T and G590A) and acetylator phenotypes (p<0.0001). However, G857A was not associated with acetylator phenotypes (p=0.340). This study reported a high frequency of children with slow acetylator status. Previous studies have reported a similar prevalent rate. This further emphasizes the need to carryout pharmacogenomic studies in order to improve on drug dosing, metabolism and individualization of response.

1584

EFFECT OF PROLONGED FLAVIVIRUS RNA SHEDDING IN CHIKUNGUNYA CHRONICITY OF INDIVIDUALS WITH ARBOVIRUS CO-INFECTIONS

Marta G. Cavalcanti, Eduardo Scarlatelli Pimenta, Mauro Jorge Cabral-Castro, Jose Mauro Peralta

Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Background: Chikungunya infection became a global public health issue. Clinical manifestations may evolve to prolonged pain, physical impairment and chronicity. In arbovírus co-transmission areas, the role of Zika (ZIKV) and Dengue (DENV) virus co-infections in the development of Chikungunya virus (CHIKV) chronic infection is still poorly understood. Methods: During 2015-2018, 87 laboratory-confirmed CHIKV, ZIKV and / or DENV mono or co-infected individuals from a cohort were followed-up. For arbovirus diagnosis, serial blood and urine samples were tested by molecular methods (RT-PCR), and sera by ELISA test for anti-CHIKV IgM/IgG detection. Results: The study population included 54 female and 33 male (mean age of 47.6 ± 5.58 years old). CHIK, ZIKV and DENV mono infections were confirmed in 34 (39.1%), 10 (11.5%) and 3 (3.5%), respectively. Co-infections (CO) were diagnosed in 32 (36.8%): being 28 (32.2%, CHIKZIKV), 4 (4.6%, CHIKDENV) and 4 (4.6%, CHIKZIKVDENV). Also, four (4.6%) individuals presented DENVZIKV CO. Total cases of CHIKCOs showed a slight increase compared to CHIKMono (mean:428,7 ± 382.5 days post-onset (dpo); 95%CI: 480-558.1 x 356.1 ±. 355.2 dpo; 95%CI: 232-480). CHIKZIKV CO RNA shedding duration differed significantly from CHIK Mono (432 ± 378.6 dpo, 95% Ci: 285.2-578.8 x 250 ±. 271.8 dpo; 95% CI: 122.8-377.1; p < 0.036). CHIKZIKVCO represented 75% of the chronic cases, and 7.14% of the cases progressed to a fatal outcome. CHIKVCO group presented a 1.109 and 2.86 folders higher relative risk for chronicity and persistent ZIKV RNA shedding in biological specimens, respectively. Conclusion: Findings suggest that flavivirus infection might be a factor associated with CHIKV infection chronicity. And, it resulted from a delayed flavivirus clearance.

1585

HOUSEHOLD CASSAVA FLOUR CYANIDE AND URINARY THIOCYANATE LEVELS ARE PREDICTIVE OF NEUROCOGNITIVE AND MOTOR PROFICIENCY DEFICITS IN SCHOOL-AGE CONGOLESE CHILDREN DEPENDENT ON TOXIC CASSAVA

Michael J. Boivin[1], Musasa Hanshi Hilaire[2], Alla Sikorskii[1], Nicole Mashukano[3], Hilaire Lufuluabo[2], Tshingamb Ndaya[4], Gregoire Kamanga[2], Banea J. Mayambu[3], Justin Kombi[2], Gedeon Bongo[4], Esperance Kashala-Abotnes[5], Daniel Okitundu[2], Dieudonne Ngoyi Mumba[4], Desire Tshala-Katumbay[6]

[1]*Michigan State University, East Lansing, MI, United States,* [2]*University of Kinshasa, Kinshasa, Democratic Republic of the Congo,* [3]*Programme National de Nutrition (PRONANUT), Kinshasa, Democratic Republic of the Congo,* [4]*Institut National de la Recherche Biomédicale (INRB), Kinshasa, Democratic Republic of the Congo,* [5]*University of Bergen, Bergen, Norway,* [6]*Oregon Health Sciences University, Portland, OR, United States*

Objective: Our group has previously identified biomarkers of neurocognitive and motor deficits associated with chronic dietary reliance on poorly processed cyanogenic cassava, a staple food crop for millions of children in the Democratic Republic of the Congo (DRC). Method: Primary outcomes were cassava flour cyanogenic content produced and urinary concentrations of thiocyanate (U-SCN) in children 5 to 14 years of age in konzo-affected households. Secondary outcomes were the neuropsychological performance of children assessed using the Kaufman Assessment Battery for Children (KABC-II) for cognitive ability and the Bruininks/Oseretsky Test (BOT-2) for motor proficiency. Results: In a sample of 361 children with konzo disease (mean age 10.2 yrs) and 343 neighbor controls without konzo (9.0 yrs), konzo children performed significantly more poorly than controls on all KABC-II and BOT-2 performance outcomes except KABC-II simultaneous processing (visual-spatial analysis/problem solving). For our present study cohorts at baseline, household flour cyanide level was predictive of child U-SCN (OR=3.58, 95% CI [1.24, 10.33], p=0.02). For konzo children, flour cyanide level and U-SCN was significantly predictive of KABC-II overall performance, sequential processing (working memory), learning, delayed recall, and planning/reasoning. For controls, household cassava flour cyanide level was only predictive of BOT-2 motor proficiency (p<0.01), and U-SCN was predictive of KABC-II sequential processing (p=0.03) and delayed recall (learning) (p=0.03). Discussion: Neurocognitive and motor proficiency deficits for the present cohort confirms our previous findings and establish the viability of monitoring household cassava flour cyanide and U-SCN at the household level for identifying communities most at risk for konzo pediatric disability. Such communities can then be prioritized for educational interventions targeting mothers/food providers, minimizing a critical toxic-nutritional risk factor for tens of millions of children throughout central and western Africa dependent on toxic cassava as their primary food staple.

1586

EFFECTS OF REMOVING USER FEES ON BARRIERS TO CARE AMONG CHILDREN WITH DIARRHEA, FEVER, AND PNEUMONIA IN RURAL MALI

Hailey Zuverink[1], Naimatou Kone[2], Mohamed Bana Traore[2], Mahamadou Sogoba[2], Caroline Whidden[2], Tracy Lin[3], Nancy Padian[3], Ari Johnson[2], Kassoum Kayentao[4], Jenny Liu[3], **Emily Treleaven**[1]

[1]*University of Michigan, Ann Arbor, MI, United States,* [2]*Muso, Bamako, Mali,* [3]*University of California, San Francisco, San Francisco, CA, United States,* [4]*Malaria Research and Training Centre, Bamako, Mali*

In settings with fractured and/or poorly resourced health systems, children and families experience a number of barriers to healthcare, contributing to excess under-five morbidity and mortality. Removal of user fees is one strategy to address cost-related barriers to care. While the effects of user fee removal on healthcare access and outcomes have been well studied, less is known about change and continuity in the types of barriers faced following removal of user fees. To identify factors inhibiting care utilization among children with common childhood illnesses, we examined change in barriers to treatment after removal of user fees using data from a cluster-randomized trial in rural Mali. From December 2016 to January 2017 and February to April 2018, mothers of recently ill children under age five were interviewed in baseline and follow-up household surveys. User fees at primary health centers and a district hospital were removed in February 2017. Respondents provided information about whether children experienced diarrhea, fever, and/or cough in the two weeks preceding the survey, as well as care utilized and barriers faced. Among 5,244 children who reported recent illness at baseline and 6,928 at follow-up, we examined frequency of self-reported barriers and test whether removal of user fees is associated with a reduction in barriers to care. The proportion of recently ill children receiving any treatment increased from 55.9% to 71.8% after user fees were removed (p=0.000). At baseline 1,556 children faced any barrier to treatment (29.7%), compared to 744 children at follow-up (10.7%), a significant reduction (p=0.000). At baseline, cost was the most frequently reported barrier (24.9%), while just 0.4% reported cost-related barriers at follow-up (p=0.000). Reporting of non-cost barriers also decreased significantly after user fees were removed (p=0.000). However, mothers were more likely to report a lack of time as a barrier to care at follow-up (0.9% vs. 4.7%, p=0.000). Removing user fees is an effective strategy to address barriers to care for children under five in this context.

1587

ANTIBIOTIC USE AMONG RESIDENTS IN UGANDA, ZIMBABWE AND MALAWI - A MIXED METHODS STUDY

Clare I. Chandler[1], Justin Dixon[1], Susan Nayiga[2], Eleanor MacPherson[3], Salome Manyau[4], Christine Nabirye[2], Mabvuto Chimenya[5], Sham Lal[4], Chrissy Roberts[1], Edward Green[3], Katharina Kranzer[1], Sarah G. Staedke[1]

[1]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [2]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [3]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [4]*Biomedical Research and Training Institute (BRTI), Harare, Zimbabwe,* [5]*Malawi Liverpool Wellcome Trust, Blantyre, Malawi*

As concerns about the prevalence of infections that are resistant to available antibiotics increase, attention has turned towards the volume of use of these medicines both within and outside of formal healthcare settings. Evidence of antibiotic use levels and how to optimise this is growing for healthcare settings. In order to inform interventions for stewardship outside of formal settings, there is an urgent need to understand which antibiotics are being used by people and to understand the reasons for this use. We developed and implemented the 'drug bag method' to capture frequency and types of antibiotics used amongst a total of 1635 households in different settings in Uganda, Zimbabwe and Malawi. We carried out observations and interviews with members of 173 households and stakeholders across the study sites to understand the significance of antibiotics in each setting. We found important differences in antibiotic use patterns between countries: metronidazole was the most self-reported 'frequently used' antibiotic in Uganda (74.4%, 95% CI 67.9-80.8), amoxycillin in Zimbabwe (57.1%, 95% CI 51.9-62.5) and cotrimoxazole in Malawi (37.6%, 95% CI 34.3-41.0). We explain these differences in terms of historical, socioeconomic and systems factors in each setting. Across countries, our data show how antibiotics have become a necessary part of household strategies for navigating inequalities in access to healthcare and the precarities of modern life. Our findings compel critical reflection on the assumption that community-level ABU can be reduced through increasing 'public awareness'. We describe how future interventions could consider systems - rather than individuals - as stewards of antibiotics, reducing the need to rely on these medicines to fix other issues of inequity, productivity and security.

1588

COMPLIANCE AND SPILLOVER EFFECTS WITH AZITHROMYCIN DISTRIBUTION FOR CHILD SURVIVAL IN NIGER: SECONDARY ANALYSES OF THE MORDOR TRIAL

Kieran S. O'Brien[1], Ahmed M. Arzika[2], Ramatou Maliki[2], Farouk Manzo[2], Alio K. Mamkara[2], Abdou Amza[3], Elodie Lebas[1], Catherine Cook[1], Benjamin Arnold[1], Catherine E. Oldenburg[1], Jeremy D. Keenan[1], Thomas M. Lietman[1]

[1]*University of California, San Francisco, San Francisco, CA, United States,* [2]*The Carter Center, Niamey, Niger,* [3]*Programme Nationale de Sante Oculaire, Niamey, Niger*

Biannual distribution of oral azithromycin to children 1-59 months old reduced child mortality by 18.1% (95% CI 10.0% to 25.5%) in the Niger site of the cluster-randomized MORDOR trial. The placebo-controlled design of this trial enabled unbiased assessment of the effect of azithromycin by subgroups of compliance with assigned treatment. Here, we compared mortality among eligible children who received treatment in azithromycin communities to those in placebo communities to determine the efficacy of the intervention in a per protocol analysis. In addition, we compared mortality among eligible children who did not receive treatment in azithromycin communities to those in placebo communities to determine the presence of spillover effects from treated to untreated children. Overall, mean treatment coverage was 90.4% (standard deviation 10.4%) across both arms during the 2-year study period. Among treated children, the mortality rate reduction with azithromycin compared to placebo was 20.2% (95% CI 11.7% to 27.9%), with mortality rates of 16.6 deaths per 1,000 person-years in azithromycin communities and 20.9 deaths per 1,000 person-years in placebo communities. Among untreated children, the mortality rate reduction with azithromycin compared to placebo was 8.8% (95% CI -20.2% to 30.9%), with mortality rates of 33.6 deaths per 1,000 person-years in azithromycin communities and 34.4 deaths per 1,000 person-years in placebo communities. Overall, untreated children experienced greater mortality in both arms. In this controlled trial setting, coverage was quite high, which is an important consideration as programs move towards implementation. This analysis shows increased efficacy among treated children and no evidence of spillover benefits to untreated children, thus high coverage will be required in programmatic implementation to ensure effects of a similar magnitude as the trial. Further analyses will elucidate factors associated with non-compliance, which could be used to identify and target vulnerable populations missed in community-based programs.

1589

THE GLOBAL BURDEN OF SNAKEBITES: A MODELING STUDY OF MORTALITY AND NONFATAL HEALTH OUTCOMES

Nicholas L. Roberts, Erin Hamilton, Theo Vos, Spencer James, David Pigott

Institute for Health Metrics and Evaluation, Seattle, WA, United States

Venomous snakebites cause significant death and disability worldwide, and were declared a Category A Neglected Tropical Disease (NTD) in 2017 by the World Health Organization. Despite the large disease burden and increased attention, there have been few efforts to quantify the global burden of venomous snakebites. This analysis expanded on estimates from the Global Burden of Disease study 2019, which estimated the burden of "venomous animal contact", an umbrella disease category that includes all venomous animals. The mortality due to venomous snakebites was modeled for every country from 1990 to 2019 by age and sex, with parameters for regional, time, and age patterns. Nonfatal disability estimates were focused in India, where the vast majority of the global burden occurs, primarily due to the "Big Four" snakes, which include the krait, cobra, Russell's viper, and saw-scaled viper. Using inputs from Indian inpatient hospital records and retrospective case series', the case fatality rate and clinical outcomes that result from venomous bites due to each snake type were simulated. In 2019, 64,395 (95% UI: 39,120 to 79,423) died globally from snakebites, which would make it the leading cause of death among NTDs. In India, 51,770 (29,575 to 64,980) died, which was almost 50,000 more deaths than in Pakistan, which had the second-most deaths. The case fatality rate of snakebites was found to be 10.1% due to kraits and cobras and 6.6% due to vipers. In 2019, venomous snakebites caused over 2.7 million disability-adjusted life years in India, a greater burden than breast cancer, HIV/AIDS, or malaria. Acute kidney failure, internal bleeding, and respiratory failure were the most significant nonfatal outcomes. This study is the first to estimate the magnitude of the mortality and disability due to venomous snakebites with a data-driven modeling approach. Through a better understanding of the epidemiology of snakebites, prevention initiatives and resources such as antivenom, dialysis machines, and ventilators can be more efficiently distributed to high-risk rural and agricultural areas and save lives that depend on quick access to care.

1590

PREVALENCE OF SCABIES AND IMPETIGO AMONGST SCHOOL CHILDREN IN TIMOR-LESTE: SCHOOL SURVEYS IN THREE MUNICIPALITIES

Alexander Matthews[1], Brandon Le[2], Paul Arkell[3], Naomi Clarke[2], Daniel Engelman[4], Merita Monteiro[5], Salvador Amaral[6], Terlinda Barros[7], Joshua Francis[8], **Susana Vaz Nery**[2]

[1]*Royal Darwin Hospital, Darwin, Australia,* [2]*Kirby Institute, Sydney, Australia,* [3]*National Health Laboratory, Timor-Leste, Timor-Leste,* [4]*Murdoch Children's Research Institute, Melbourne, Australia,* [5]*Ministry of Health, Dili, Timor-Leste,* [6]*Maluk Timor, Dili, Timor-Leste,* [7]*Hospital Nacional Guido Valadares, Dili, Timor-Leste,* [8]*Menzies School of Health Research, Darwin, Australia*

Scabies and impetigo are endemic in many tropical, low- and middle-income countries, including Timor-Leste where there is limited epidemiological data. Ivermectin is active against scabies but there is little understanding of the impact of ivermectin, diethylcarbamazine and albendazole (IDA) mass drug administration (MDA) programs for lymphatic filariasis on the prevalence of scabies. A cross-sectional school survey was conducted in April-May 2019, prior to IDA MDA by the Timor-Leste Ministry of Health. Infants and children less than 20 years of age attending six primary schools in an urban (Dili) and two rural (Ermera and Manufahi) municipalities were eligible to participate. 1043 participants were interviewed and examined by trained local and international health workers to clinically diagnose scabies and impetigo. Mixed-effects logistic regression models were used to analyse odds of infection with scabies and impetigo in each municipality accounting for clustering at school level. The population attributable risk of scabies as a cause of impetigo was also estimated. Overall unweighted prevalence of scabies was 33.4%. Children in Manufahi were 3.5 times more likely to have scabies than in Dili (53.6% vs 28.2%, AOR 3.5). Most participants with scabies (68.8%) had mild scabies (three to 10 lesions) distributed on more than one body region (64.7%). Overall unweighted prevalence of impetigo was 12.5%. Relative to Dili, children in Ermera (14.9% vs 8.7%, AOR 1.9) and Manufahi (18.0% vs 8.7%, AOR 2.2) were twice as likely to have impetigo. Scabies and impetigo prevalence were not significantly different between age groups. Impetigo was more common in children with scabies than those without (18.1% vs 9.6%, AOR 2.0), corresponding to an attributable risk of scabies as a cause of impetigo of 46.7%. Scabies and impetigo prevalence are higher than previously reported in Timor-Leste, with a greater burden of disease in rural than urban municipalities. Scabies infestation was strongly associated with impetigo. Comprehensive control strategies are urgently needed in Timor-Leste.

1593

DRUG DEVELOPMENT FOR THE TREATMENT AND CONTROL OF ONCHOCERCIASIS: POPULATION PHARMACOKINETIC AND ADVERSE EVENTS MODELING OF EMODEPSIDE (BAY 44-4400) IN HEALTHY VOLUNTEERS

Frauke Assmus[1], Richard Hoglund[1], Ivan Scandale[2], Frédéric Monnot[2], Sabine Specht[2], Joel Tarning[1]

[1]*Mahidol-Oxford Tropical Medicine Research Unit, Mahidol University, Bangkok, Thailand,* [2]*Drugs for Neglected Diseases initiative, Geneva, Switzerland*

To accelerate the progress towards elimination of onchocerciasis, a macrofilaricidal drug is urgently needed. Emodepside has shown macrofilaricidal activity and is currently under clinical development for the treatment of onchocerciasis. The aim of this study was to characterize the relationship between emodepside plasma exposure and adverse events in healthy male subjects and to propose a dosing regimen for a planned phase II clinical trial. Plasma concentration-time profiles and adverse event data were obtained from 142 subjects enrolled in three phase I studies, including a single-dose, and a multiple-dose, dose-escalation study as well as a relative bioavailability study. Following the development of a pooled population pharmacokinetic model for emodepside, logistic exposure-response regression models were fitted to safety outcomes, i.e. drug-related treatment-emergent adverse events (TEAEs) of interest reported in 27 subjects (19.0%). Both eye disorders (e.g. visual impairment, blurred vision) and nervous system disorders (e.g. dizziness, headache) were taken into account (14.1 % and 12.7% of subjects, respectively). Overall, emodepside was safe in the investigated dose groups. All drug-related TEAEs were transient, and mild or moderate in intensity. Maximum emodepside plasma concentrations (Cmax) were identified as a better TEAE of interest predictor as compared to dose or area under the concentration-time curve. Subjects with higher Cmax exposures showed a higher probability of experiencing a drug-related TEAE of interest (0.77% increase in the odds per 1 ng/mL increase in Cmax). The occurrence of eye disorders was more strongly associated with Cmax as compared to nervous system disorders. The population pharmacokinetic - adverse events model was used to derive a body-weight based dosing regimen, i.e. twice daily dosing of emodepside for 10 days, as tablet formulation in fasted state. Our results suggest that the proposed dosing regimen allows for the achievement of target exposures (> 5 days above 100 ng/mL) while maintaining acceptable tolerability margins (< 50 % predicted probability of TEAEs of interest).

1594

COMPARISON OF FOUR LONGITUDINAL OUTCOME MEASURES FOR LIMB LYMPHEDEMA

Philip Budge[1], Lalindi De Silva[2], Michael Weiler[3], Sarah Sullivan[4], Mirani Weerasooriya[2], Sharmini Gunawardena[5], Channa Yahathugoda[2]

[1]*Washington University in St. Louis, St. Louis, MO, United States,* [2]*University of Ruhuna, Galle, Sri Lanka,* [3]*LymphaTech, Atlanta, GA, United States,* [4]*Task Force for Global Health, Atlanta, GA, United States,* [5]*Antifilariasis Campaign, Colombo, Sri Lanka*

Limb lymphedema from lymphatic filariasis (LF) or podoconiosis is a major cause of chronic disability worldwide. Improving lymphedema care is a pillar of the Global Programme to Eliminate LF, and clinical trials of new therapies are needed. Outcome measures for such trials should be clinically relevant, informative, and sufficiently sensitive and reproducible to enable meaningful comparisons. The LEDoxy Study is an international, prospective, randomized, placebo-controlled trial of the effect of doxycycline on limb lymphedema of presumed filarial origin. Study outcomes include (1) change in clinical (Dreyer) stage, (2) limb volume and circumference measured by portable 3D infrared imaging (3DII), (3) limb circumference by tape measurements, and (4) skin thickness measured by ultrasound (STU). We compared these four outcome measures in 219 participants from the still-blinded study site in Sri Lanka. Among 200 participants with stage 1-3 limb lymphedema at baseline, 182 (91%) were present for follow up at 12 months. Among these, 66 (36%) evidenced a reduction in clinical stage, while 100 (55%) had no change and 16 (9%) progressed to a higher clinical stage. Limb size was smaller by at least 100 mL for 30 (17%) participants and larger by at least 100 mL for 44 (24%) participants. Change in limb size did not always correlate with changes in stage. Among those with a reduced clinical stage at 12 months, 77% had equivalent or lower limb volumes, but 23% had increased limb volume. Median % difference between duplicate measurements was 0.0 (IQR 0.0 - 0.3) for tape measurements, 1.1 (IQR 0.5 - 2.1) for 3DII volumes, and 3.7 (IQR 2.0 - 6.9) or 5.7 (IQR 2.0 - 8.5) for STU over the medial or lateral malleoli, respectively. There was good correlation between limb measurements taken by 3DII vs. tape measure ($r^2 > 0.92$), but poor correlation between circumference changes and STU (r^2=0.16 to 0.25). Conclusions: Measurements of limb circumference are more reliable than STU in monitoring skin thickness changes. Changes in stage are partially correlated with anthropometric changes, suggesting that both should be included in lymphedema outcome trials.

1595

THE SAFETY OF TRIPLE DRUG TREATMENT WITH IVERMECTIN, DEC, AND ALBENDAZOLE IN PERSONS WITH ONCHOCERCIASIS

Nickolas O. Opoku[1], Michael E. Gyasi[1], Eric M. Kanza[1], Augustine R. Hong[2], Catherine M. Bjerum[3], Christopher L. King[3], Peter U. Fischer[4], Gary J. Weil[2]

[1]*University of Health and Allied Sciences, Hohoe, Ghana,* [2]*Washington University School of Medicine, St. Louis, MO, United States,* [3]*Case Western Reserve University, Cleveland, OH, United States,* [4]*Washington University in St. Louis, St. Louis, MO, United States*

Recent studies have shown that triple drug treatment with ivermectin plus DEC and albendazole (IDA) is superior to two-drug regimens for treatment of lymphatic filariasis (LF). IDA is not approved for use by LF elimination programs in countries with onchocerciasis because of safety concerns. The present study was performed in eastern Ghana to assess the safety of IDA in persons with onchocerciasis after pretreatment with ivermectin. Eligible participants [ages 16-70 yr with microfiladermia (&lt 3 Mf/mg) and &lt 1 palpable onchocercoma] were pretreated with ivermectin to reduce Mf counts in skin and eyes. Participants were retreated with ivermectin 6 to 12 mo later and then randomized to receive either a single oral dose of ivermectin plus albendazole (IA, 44), a single dose of IDA (45), or three daily doses of IDA (44). Complete safety assessments were performed on day 3, day 7, and 3 months after treatment. This included general physical examinations plus tests of visual acuity, visual fields, slit lamp examinations, indirect ophthalmoscopy, retinal photography, and ocular coherence tomography. 133 participants have been treated and completed day 7 post-treatment safety assessments to date. Approximately 30% of participants experienced adverse events (AE) after treatment (26% mild, 4% moderate, 0% severe or serious). This AE rate is lower than the 80% AE rate observed after ivermectin pretreatment. The most common AE were Mazzotti reactions with pruritis, rash, and headache. Five participants had mild ocular AE (most commonly periocular itching and one small intraretinal hemorrhage. AE were more common in men than in women, and AE rates were not signficantly higher after IDA (31.3%) than after IA alone (27.3%). Unfortunately, the study is on hold now due to the COVID-19 pandemic. We hope to resume enrollment soon so that we can expand the safety database and assess the impact of IDA on *O. volvulus* adult worms (18 months after treatment). Preliminary results reported here suggest that IDA is safe for patients with onchocerciasis after ivermectin pretreatment. This strategy could expand use of IDA to accelerate LF elimination in Africa.

1596

DEVELOPMENT AND ASSESSMENT OF THE PSYCHOMETRIC PROPERTIES OF A NEW SCALE (15 ITEM PSB-CL) TO MONITOR THE PSYCHOSOCIAL BURDEN OF CHRONIC LYMPHEDEMA IN FILARIASIS

Janaka Ruben, **Thishan Channa Yahathugoda**, Mirani Vasanthamala Weerasooriya, Chandanie Senadheera, K. G. Somasiri, Bilesha Perera

Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka

Chronic lymphedema (CL) of lymphatic filariasis causes severe psychosocial burdens (PSB) to its victims. Currently established scales to measure PSB of CL are not culturally adaptable to Sri Lankan patients. In this study, we developed and assessed the psychometric properties of a disease-specific questionnaire (15 item PSC-CL). A detailed literature search was done to identify scales related to PSB of CL patients. Questions were developed through collected expressions from semi-structured interviews with 46 CL patients. A response scale was developed from the collected pool of possible responses, and they were scored using a visual analogue scale. Face and content validity were carried out using an initial questionnaire (31 questions). They were reduced to 27 by removing unclear, ambiguous, double-stranded ones and those with value-laden words. This new questionnaire was administrated on 92 patients by a group of doctors for item reduction by the analysis of internal consistency (Cronbach's alpha ≥0.7) and factor analysis (Principal Component Analysis; Eigenvalues ≥ 1). After patient survey and factor analysis, the final questionnaire PSB-CL was formed with 15 questions that had four subscales (factors), physical, social, fear and humiliation. Patients (n=92) reported quite a large percentage (42% - 69%) of problems under each dimension. The scale promises to be a good tool to measure the psychosocial burden of chronic lymphedema in lymphatic filariasis patients in Sri Lanka. Further validation of the tool is needed.

1597

USING MOBILE DATA COLLECTION TECHNOLOGY TO HELP OVERCOME CHALLENGES DURING MASS DRUG ADMINISTRATION (MDA) IN PORT-DE-PAIX, HAITI

Alain Javel[1], Eurica Denis[1], Carl Renand Fayette[1], Paul-Emile Dalexis[1], Marc-Aurele Telfort[2], Ellen Knowles[3], Mary Linehan[3], Abdel Direny[3], Josh West[4], Benjamin Crookston[4]

[1]*IMA World Health, Port au Prince, Haiti,* [2]*Ministry of Health and Population, Port au Prince, Haiti,* [3]*IMA World Health, Washington, DC, United States,* [4]*Brigham Young University, Provo, UT, United States*

Since 2000, much progress has been made in Haiti to eliminate lymphatic filiaraisis (LF) as a public health threat. Port-de-Paix is one of the communes that have twice failed the pre-transmission assessment survey (pre-TAS) and continues to implement mass drug administration campaigns. In 2019, the Haiti Neglected Tropical Disease Control Program (HNTDCP) used Open Data Kit (ODK), a real time data collection tool, to improve data quality and collect additional information related to MDA in Port-de-Paix. Eighteen community health workers (CHWs) were trained to use ODK on smartphones and then collected data during MDA supervision. Each CHW was assigned at least two distribution posts to collect data on GPS coordinates, supervision checklists, and disaggregated treatment by sex, age and subdistrict, which were then sent to a central server for analysis and quick decision making. The CHWs sent 1889 forms for all 199 distribution posts. Results showed that at least 6 localities were not covered while 26% of the distribution posts were located at less than 50 meters from each other in some areas. The data also revealed that some distribution posts were located outside the commune boundaries. With this information, community leaders were able to follow up during MDA to facilitate real-time program adaptation and to reach uncovered areas. By using this tool, CHWs were able to identify 20% of distribution posts that were without a functional megaphone, which is important for social mobilization during MDA. The CHWs also found that in 12% of the distribution posts, registration forms were not completed properly on the first day of MDA. By the last day of the campaign, the rate was reduced to 1.6% thanks to on-site training of the community drug distributors (CDDs). Because the data were analyzed daily, the HNTDCP provided appropriate recommendations to supervisors and CDDs to resolve issues. The experience of Port de Paix showed how real-time mobile data collection with ODK can improve the MDA coverage and quality reporting. The HNTDCP plans to extend the use of this tool in other communes in order to decrease LF transmission and to reach its elimination goal by 2030.

1598

APPLYING THE RANAS FRAMEWORK TO ASSESS PSYCHOSOCIAL DRIVERS OF COMPLIANCE WITH MASS DRUG ADMINISTRATION FOR LYMPHATIC FILARIASIS

Caitlin M. Worrell[1], Christiana Titaley[2], Ryan Wiegand[1], Filda de Lima[2], Yuniasih Taihuttu[2], Tara A. Brant[1], Lita Renata[3], Alison Krentel[4]

[1]*The Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Faculty of Medicine, Pattimura University, Ambon, Indonesia,* [3]*Subdit Filariasis, Ministry of Health, Jakarta, Indonesia,* [4]*Bruyère Research Institute, Ottawa, ON, Canada*

High community participation in public health campaigns, such as mass drug administration (MDA) for lymphatic filariasis (LF) is critical for achieving global elimination targets. Despite numerous rounds of MDA, many programs lack information on what motivates community participation in MDA campaigns. We aimed to adapt the risks, attitudes, norms, abilities, and self-regulation (RANAS) framework to determine if it could detect differences in psychosocial factors between LF MDA compliers and non-compliers. In 2018, a cross-sectional survey of residents was conducted in Air Salobar and Waihaong in Ambon City, Indonesia. At each site, 20 sub-hamlets with probability proportional to estimated size and 25 and 23 households per sub-hamlet were selected randomly in Air Salobar and Waihaong respectively. Individuals were asked if they were aware of the MDA, received pills (coverage), and swallowed the pills (compliance). Behavioral variables, based on the RANAS model, were measured on a 5-point Likert scale. Behavioral variables were filtered and minimized by lasso regression to select factors influencing compliance; selected variables were fit to a logistic regression model to generate odds ratios (OR). A total of 995 individuals were enrolled with 495 from Air Salobar and 500 from Waihaong. Among respondents, 95.4% (n=951) were aware of the MDA, 69.2% (n=690) received the pills, and 36.7% (n=366) swallowed the pills. Compared with compliers, non-compliers had increased odds of difficulty swallowing pills (OR=1.42; 95% confidence interval (CI): 1.15, 1.76), reduced confidence in understanding instructions for swallowing pills (OR=1.80; CI: 1.41, 2.23), lower family MDA participation (OR=2.47; CI: 2.15, 2.83), and fewer reports of swallowing pills automatically (OR=1.56; CI: 1.29, 1.89). Study results were used to draft a new approach to community awareness that addressed the predominant psychosocial drivers of compliance. While factors influencing community participation in MDA campaigns varies by site, structural behavioral models may help programs quickly identify and respond to psychosocial drivers of participation.

1599

ASSESSING ANTI-FILARIAL ANTIBODY AS A COMMUNITY INFECTION INDICATOR IN AREAS TREATED WITH DOUBLE- OR TRIPLE-DRUG MASS DRUG ADMINISTRATION IN QUARTIER MORIN, HAITI

Keri L. Robinson[1], Christine L. Dubray[1], Anita D. Sircar[1], Jean Romuald Ernest[2], Marisa Hast[1], Ryan Wiegand[1], Jean Frantz Lemoine[3], Kimberly Won[1]

[1]*Centers for Disease Control and Prevention, Atlanta, GA, United States,* [2]*Pan American Health Organization, Port-au-Prince, Haiti,* [3]*Misnistere de la Sante et de la Population, Port-au-Prince, Haiti*

Mass drug administration (MDA) with albendazole plus either diethylcarbamazine (DEC) or ivermectin has been the standard strategy for lymphatic filariasis elimination. MDA with all three (IDA) was recently introduced after evidence suggested longer sustained clearance of microfilaremia (Mf) and some macrofilaricidal effect. Currently, program decisions are made by measuring Mf and circulating filarial antigen (CFA). Tools like anti-filarial antibody (Ab) assays are increasingly available but their ability to detect a population level change must first be evaluated. In Haiti, we compared prevalence of the anti-filarial Ab anti-Wb123 between 5 localities that received DEC and albendazole (DA) and 5 that received IDA. At baseline, 5,993 participants (≥5 years) were enrolled and tested for CFA by filariasis test strip; only CFA positive participants were examined for Mf. Dried blood spots (DBS) were prepared for Ab testing. A subset of baseline DBS was randomly selected to assess presence of Ab by multiplex bead assay. One year later, randomly selected participants in the same 10 localities were assessed for treatment impact. In the DA arm 117/999 (11.7%), 21/117 (17.9%) and 292/978 (29.9%) were positive for CFA, Mf and Ab, respectively. In the IDA arm 80/1001 (8.0%), 12/80 (15.0%) and 156/974 (16.0%) were positive for CFA, Mf and Ab, respectively. At follow up, assessed biomarkers in the DA arm decreased to 10.0% (CFA), 16.0% (Mf) and 22.7% (Ab). The Ab decline was significant ($p < 0.01$). Biomarkers in the IDA arm increased to 8.5% (CFA), 20.2% (Mf) and 19.7% (Ab), but differences were not significant. There was better treatment compliance in the DA arm (53.6% DA, 46.2% IDA; $p < 0.001$) based on self-reported participation. Microfilaremia significantly decreased among those who took the drugs (DA $p < 0.001$, IDA $p < 0.05$), but CFA and Ab did not decrease. While Ab significantly changed in the DA arm, neither arm reached 65% recommended MDA coverage. The current study does not provide enough evidence to draw conclusions on the utility of antibody tools in this setting. Further evaluation with additional time points and better MDA compliance should be pursued.

1600

KNOCKOUT OF IMMUNE GENES IN *ANOPHELES STEPHENSI* USING CRISPR CAS9 REVEALS THEIR ROLE IN INFECTION PERMISSIVENESS AND REPRODUCTIVE CAPACITY

Ehud Inbar[1], Abraham Eappen[1], Robert Alford[2], Robert Harrel[2], William Reid[3], Tao Li[1], Sumana Chakaravarty[1], Donald Ward[1], Maryam Hosseini[1], Kim Lee Sim[1], Stephen L. Hoffman[1], Peter F. Billingsley[1]

[1]*Sanaria Inc., Rockville, MD, United States,* [2]*University of Maryland, Rockville, MD, United States,* [3]*University of Missouri, Columbia, MO, United States*

Sanaria® PfSPZ Vaccine and PfSPZ-CVac are protective against *Plasmodium falciparum* (Pf) infections. These vaccines are composed of aseptic, purified, cryopreserved Pf sporozoites (SPZ), and are manufactured using aseptically grown *Anopheles stephensi* mosquitoes. Previous studies showed that *Anopheles* immune response genes, such as the Leucin-Rich Immune (LRIM) protein and Thioester-Containing Protein 1 (TEP1) inhibit *Plasmodium* sporogony by melanization and subsequent phagocytosis of the developing oocysts. Thus, knocking out these genes should theoretically increase PfSPZ infection intensities and thereby improve manufacturing efficiency of PfSPZ products. We have knocked out three immune genes: LRIM1, TEP1, and the LPS-induced TNF alpha transcription factor-like 3 (LL3). The knockouts were confirmed at the DNA, RNA and protein levels. We have previously reported on the surprising outcomes of LRIM1 knockout on different aspects of the mosquito's biology. Deletion of LRIM1 significantly altered the microbiome composition and life span of the mosquitoes. Contrary to our expectations, in non-aseptic conditions, the absence of LRIM1 did not improve the infection but nearly abolished it. The restoration of the infection by antibiotics or by growing the mosquitoes aseptically suggest that the LRIM1 is crucial for Pf infection by regulating the internal mosquito microbiota, allowing a permissive environment in which the parasite can prosper. In line with these observations, knockout of TEP1, another member of the complement-like system, to a lesser extent also resulted in decreased longevity, reproductive capacity and ability to support Pf infection. Surprisingly, deletion of LL3 did not significantly affect Pf infection; the effect on mosquito reproduction is under investigation. Collectively, our results suggest that immune genes have a more agonistic rather than antagonistic effect on parasites when in mosquitoes, and shed light on the interplay between immunity and reproduction in the mosquitoes.

1601

CTL4-KNOCKOUT TO SUPPRESS *PLASMODIUM* TRANSMISSION IN THE VECTOR MOSQUITO

Maria L Simoes, Yuemei Dong, Godfree Mlambo, George Dimopoulos

Johns Hopkins University, Baltimore, MD, United States

The development of genetically modified (GM) mosquitoes for malaria control has gained strength through the recent advances in gene-drive technology and an increased understanding of vector-parasite interactions. While most work aiming at the development of GM malaria resistant mosquitoes, suitable for population replacement, has focused on the over-expression of anti-parasitic genes, here we have explored a strategy relying on CRISPR/Cas9-based inactivation of mosquito-encoded *Plasmodium* agonists. During its sexual cycle inside the mosquito vector, *Plasmodium* engages in intimate interactions and relies in numerous *Anopheles*-derived host factors, which act as facilitators of infection. The C-type lectin CTL4 has been identified previously as a *Plasmodium* agonist. In our recent work we showed that the C-type lectin-mediated protection against parasite melanization in the African vector *A. gambiae* is dependent on the intensity of infection, rather than the mosquito-parasite combination. RNA interference (RNAi)-based silencing of CTL4 resulted in melanization and reduction of live parasites, albeit RNAi results in only partial gene silencing. We hypothesized that the knockout (KO) of CTL4 would result in complete melanization of the parasites, and consequently a complete halt of *P. falciparum's* development inside the mosquito. We are currently using CRISPR/Cas9 technology for targeted CTL4-KO in *A. gambiae*. We generated gRNA overexpressing *A. gambiae* transgenic lines that were crossed with the Vasa::Cas9 strain, to generate CTL4-KO *A. gambiae* mutants. These CTL4-KO GM mosquitoes are being evaluated for parasite blocking of total *Plasmodium*-agonist disruption. The effects of CTL4-KO on bacterial and fungal development inside the mosquito are also being screened.

1602

ENGINEERING TRANSGENIC *AEDES AEGYPTI* RESISTANT TO ARBOVIRUS TRANSMISSION

Adeline E. Williams[1], William R. Reid[2], Irma Sanchez-Vargas[1], Alexander W. Franz[2], Malcolm J. Fraser[3], Jingyi Lin[2], Ken E. Olson[1]

[1]*Colorado State University, Fort Collins, CO, United States,* [2]*University of Missouri, Columbia, MO, United States,* [3]*University of Notre Dame, South Bend, IN, United States*

The mosquito vector *Aedes aegypti* transmits medically relevant arboviruses, including Zika (ZIKV), dengue (DENV), and chikungunya (CHIKV). Few vaccines exist and therapeutic interventions are limited. Controlling mosquito populations is the best method to prevent transmission, but this strategy traditionally relies on long-term insecticide

use, which is expensive and leads to resistance. Novel mosquito control strategies, including genetically manipulating these vectors, are being developed as additional tools to combat arbovirus transmission. Using CRISPR/Cas9, we have developed transgenic *Ae. aegypti* lines that express several anti-viral effector genes. For example, we developed transgenic *Ae. aegypti* that express an anti-viral group-1 ribozyme targeting the highly conserved 5′ circularization sequences present in ZIKV, DENV, and CHIKV. This strategy involves splicing the viral genome to an apoptosis-encoding gene, resulting in the elimination of infected cells. We have also developed transgenic *Ae. aegypti* that, after a bloodmeal, transcribe inverted repeat (IR) RNA sequences targeting the ZIKV and DENV2 genomes, which triggers the endogenous small-interfering RNA (siRNA) anti-viral pathway. We have confirmed expression of transgenic cargo *in vivo,* and we have challenged these mosquitoes with several strains of ZIKV, DENV2, and CHIKV to assess resistance; we are currently quantifying infection prevalence and virus titers. We are also testing the efficacy and stability of these effector cargos through CRISPR/Cas9-mediated knock-in targeting several genomic loci.We plan to couple the most efficacious anti-viral cargo with a CRISPR/Cas9-mediated gene drive element. These transgenic *Ae. aegypti* could be a significant tool to combat vector-borne diseases through a population-replacement approach.

1604

HUNTING SEXUAL STAGES OF *PLASMODIUM FALCIPARUM* PROTEINS TO IDENTIFY TRANSMISSION BLOCKING TARGETS AGAINST MALARIA

Guodong YC Niu

Florida International University, Miami, FL, United States

Concerns about the increased insecticide resistance and the recently emerging resistance of *Plasmodium* parasites to artemisinin medicines have prompted researchers to find new strategies to stop the spread of malaria. Among them, malaria transmission-blocking vaccines and drugs are the most studied, but no effective reagents have been developed yet. Infectious parasites, ookinetes, are formed in mosquitoes and need to interact with midgut proteins to penetrate the peritrophic matrix (PM) and the midgut epithelial cells. Next in midgut basal cells, the ookinetes are developed into oocysts, which release sporozoites and the sporozoites migrate to the salivary glands and infect the next person by a bite. The interaction between parasites and mosquito midgut is a key factor in a successful infection. To identify the transmission-blocking targets against malaria, we began to study the interaction between the sexual stages of the parasite proteins and the midgut of the mosquito. According to the following criteria: it contains a signal peptide, and its expression in the ookinete stage and gametophyte V stage is ≥ 5 times higher than the asexual stage (ring), we selected 99 proteins from *Plasmodium falciparum*. We first amplified the genes and expressed them using the baculovirus expression system, and then successfully expressed 78 genes at detectable levels. Next, we conducted an ELISA analysis to study the parasite-mosquito interaction and determined that 26 proteins bind to the mosquito midgut. In addition, the biological characteristics of the identified proteins support their association with the invasion of parasites in mosquitoes, such as the 61% (16/26) proteins rich in the apicoplast, the relatively small size, and more than 1/3 of the proteins specific for PM binding. We further analyzed their knockout phenotypes. Among them, PF3D7_1204400 (Pfs37) and PF3D7_0406200 (Pfs16) are very promising as potential targets for further research. Finally, we generated polyclonal antibodies to study their effects on the development of *P. falciparum* in mosquitoes.

1605

GLYCOLYSIS AND BLOOD-MEAL ENHANCED IMMUNITY OF *ANOPHELES GAMBIAE* MOSQUITOES

Alex S. Moon, Jiannong Xu

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

Mosquitoes have evolved an effective innate immune system to cope with various immune challenges presented throughout its life. A female must take a blood meal to promote egg production, and for a pathogen, a blood meal provides a remarkable avenue to gain access to the mosquito. The mosquito's immune system requires energy and metabolic support to prevent pathogen development. We posited the blood-fed female's immunity is based on the metabolic infrastructure. We found blood-fed females have a better survival than sugar-fed females when challenged with a bacterial injection of *Enterobacter*. This suggests an enhanced immunity post blood meal ($p < 0.001$). This blood-meal enhanced immunity is GAPDH activity dependent, because when GAPDH is inhibited by an inhibitor the antibacterial immunity becomes compromised. GAPDH plays an important role in glycolysis, as well as non-glycolysis functions. Interestingly, a blood meal downregulates GAPDH transcription and protein levels, which was demonstrated by transcriptome analysis and western blotting. RNAseq data showed a decrease in GAPDH transcripts for injury and bacterial injection. Further investigation is warranted to define the role of GAPDH, glycolysis in mosquito immunity.

1606

REVISING THE PROCESS OF SEX DIFFERENTIATION IN THE *ANOPHELES GAMBIAE* MOSQUITO USING RNA INTERFERENCE FOR *SEXLETHAL* AND *DOUBLESEX*

Mabel L. Taracena[1], Catherine M. Hunt[1], Mark Q. Benedict[1], Pamela M. Pennington[2], Ellen M. Dotson[3]

[1]*CDC Foundation, Atlanta, GA, United States,* [2]*Universidad del Valle de Guatemala, Guatemala City, Guatemala,* [3]*Centers for Disease Control and Prevention, Atlanta, GA, United States*

Genes involved in female differentiation have been suggested as targets for RNA interference (RNAi) to eliminate females from a cohort as a part of novel vector control programs. In diptera, *sex-lethal (sxl)* is the only gene in the sex-differentiation pathway known to be required for initiating the process and maintaining sex-specific cell commitment by gene expression control in sexually dimorphic tissues. It sets off down-stream genes to the female state, including *transformer*, *transformer-2*, *doublesex* (*dsx*), and *fruitless*. In *Anopheles gambiae*, functional characterization of most of these genes is scarce. We previously showed that the female isoform of *dsx* (*dsxf*) is overexpressed in salivary glands and ovaries and feeding dsRNA to adults affects reproductive parameters and skews progeny sex ratio. We analyzed expression patterns of *Ag sex-lethal* isoforms (*Agsxl*-RA and *Agsxl*-RB) and used RNAi to assess its use for female elimination together with *dsxf*. In contrast to *Drosophila,* we confirmed that these isoforms are expressed in different tissues across life stages and sexes. *Agsxl*-RA expression had a parallel pattern to *dsxf*, suggesting their involvement in maintaining female-specific dimorphisms in salivary gland and ovaries. For RNAi, we fed adult mosquitoes with sugar-water containing dsRNA for *sxl*-RA or *sxl*-RB, with or without dsRNA for *dsxf,* prior to blood-feeding. We measured significant reductions of mRNA levels of both isoforms in male and female whole body, 48 hours after the blood meal (80% reduction of *Agsxl*-RA in males and 65% in females; 50% reduction of *Agsxl*-RB in males and 73% in females). When silenced alone, we did not observe a, significant impact on reproductive parameters. When silenced together with *dsxf*, substantial reductions in biting efficiency (35%), egg production (70%), and progeny sex ratios (30% fewer females) were observed. To achieve female elimination, further understanding of the pathway is necessary and our method of RNAi feeding ilencing in adults will require additional is both an important tool for gene exploration and possible a viable method to improve sex sorting strategies.

1607

IMMUNOLOGIC PROFILES DEFINING CLINICAL STATES IN MALARIA

Maureen Ty[1], Aditya Rao[1], Kathleen Dantzler[1], Lauren De La Parte[1], Felistas Nankya[2], Kenneth Musinguzi[2], Isaac Ssewanyana[2], Rek John[2], Holden Maecker[1], Margaret Feeney[3], Grant Dorsey[3], Bryan Greenhouse[3], Moses Kamya[2], PJ Utz[1], Purvesh Khatri[1], Bali Pulendran[1], Prasanna Jagannathan[1]

[1]*Stanford University, Stanford, CA, United States,* [2]*Infectious Diseases Research Collaboration, Kampala, Uganda,* [3]*University of California, San Francisco, CA, United States*

Children repeatedly exposed to *Plasmodium falciparum (Pf)* malaria eventually develop naturally acquired immunity, characterized by a decline in symptomatic malaria episodes and an increase in asymptomatic *Pf* infections. To better characterize the molecular networks underlying the clinical phenotypes of malaria infection, we performed in depth characterization of transcriptional, phenotypic, functional, and epigenetic characteristics of peripheral blood among Ugandan children experiencing symptomatic or asymptomatic malaria infection, in comparison with an uninfected timepoint. Whole blood transcriptome analysis revealed 1004 differentially expressed genes comparing children with symptomatic malaria infection with an uninfected baseline. Pathways associated with IFNγ, type1 IFN and complement cascade were upregulated in these children. Surprisingly, there were no differentially expressed genes comparing children that are asymptomatic to uninfected children. Using CyTOF, we found that children with symptomatic infection had an enrichment of activated B cells and less myeloid-like cells compared with either asymptomatic or an uninfected timepoint. Interestingly, following *in vitro* stimulation with the parasite, children with asymptomatic infection had higher frequencies of IL-21-producing CD4 T cells compared to symptomatic or uninfected timepoints. Using mass cytometry with epigenetic profiling, we identified differential dimethylarginine modifications in effector memory CD4 T cells and ubiquitylation of histone H2b at lysine residue 120 in Vd2 gd T cells between individuals with asymptomatic infection compared with symptomatic infection or an uninfected timepoint. Together, these data suggest that cell-specific epigenetic modifications and functional capacity may distinguish the immune phenotype of individuals experiencing asymptomatic malaria infection. Elucidating the mechanisms and dynamics of these cellular changes longitudinally will be crucial to better understand naturally acquired immunity and may have implications for future vaccine development and control interventions.

1608

IDENTIFYING TARGETS OF FUNCTIONAL ANTIBODIES THAT PROTECT AGAINST MALARIA TO ACCELERATE VACCINE DEVELOPMENT

Linda Reiling[1], Michael T. White[2], Daniel H. Opi[1], Gaoqian Feng[1], Liriye Kurtovic[1], Freya J. Fowkes[1], Ivo Mueller[2], James G. Beeson[1]

[1]*Burnet Institute, Melbourne, Australia,* [2]*Institute Pasteur, Paris, France*

The currently most advanced vaccine candidate against *Plasmodium falciparum* malaria has limited efficacy of up to 50%. To improve on this, a next-generation vaccine will most likely have to comprise several antigens to induce a strong and protective immune response. So far, one of the biggest limitations in vaccine development has been the lack of functional correlates of protection for blood-stage infections. Growth inhibition assays mostly measure direct invasion inhibition and have not been consistently associated with protection. We have developed novel *in vitro* assays that enable us to examine functional immune responses in exposed children and adults and to assess their role in protective acquired immunity. We have identified complement-fixation as a new correlate of protection for acquired immunity that can be used to quantify functional antibodies to multiple antigens across the life cycle. We propose that these approaches generate important data to inform future vaccine design and to assess vaccine-induced immunity. We found that the presence of antigen-specific complement-fixing was strongly predictive of protection against clinical disease, and in some cases associated with controlling high-density parasitemia. By using mathematical modeling approaches, we further identified combinations of complement-fixing antibody responses against merozoite antigens that are predicted to increase protection to near 100%. In order to fill further gaps in the knowledge about which antigen combinations and functional responses are the strongest predictors of efficacious protection, we have extended our mathematical modeling to characterize combinations of total IgG as well as immunoglobulin subclass responses IgG1 and IgG3. Lastly, we aim to model the magnitude of protective effects by combining complement-fixing effect with other downstream functional mechanisms as Fc Receptor-engaging antibody responses.

1609

NATURALLY ACQUIRED HUMAN MONOCLONAL ANTIBODIES BLOCK *PLASMODIUM FALCIPARUM* TRANSMISSION TO MOSQUITOES

Amanda Fabra Garcia[1], Roos M. de Jong[1], Marta Szabat[2], Stefanie Vogt[2], Aoise O´Neill[2], Stephanie Pfaffen[2], Sherie Duncan[2], Veronique Lecault[2], Carl Hansen[2], Karina Teelen[1], Rianne Stoter[1], Marga van de Vegte-Bolmer[1], Geert-Jan Van Gemert[1], Judith Bolscher[3], Tonnie Huijs[3], John Rek[4], Margaret Feeney[5], Bryan Greenhouse[6], Rick King[7], Randall MacGill[7], Robert Sauerwein[8], Teun Bousema[1], Matthijs Jore[1]

[1]*Radboudumc, Nijmegen, Netherlands,* [2]*AbCellera, Vancouver, BC, Canada,* [3]*TropIQ Heath Science, Nijmegen, Netherlands,* [4]*Infectious Disease Research Collaboration, Kampala, Uganda,* [5]*Department of medicine, University of California San Francisco and department of pediatrics, University of California San Francisco, San Francisco, CA, United States,* [6]*Department of medicine, University of California San Francisco, San Francisco, CA, United States,* [7]*PATH Malaria Vaccine Initiative, Washington, DC, United States,* [8]*Radboudumc and TropIQ Heath Science, Nijmegen, Netherlands*

Malaria, caused by *Plasmodium* parasites, affects hundreds of millions of people every year, resulting in hundreds of thousands of deaths and a significant economic burden. The spread of the parasite occurs through bites of infected *Anopheles* mosquitoes. Blocking parasite development inside the mosquito vector is a fundamental strategy towards malaria eradication. We have recently demonstrated that naturally acquired antibodies against the two sexual stage proteins, Pfs48/45 and Pfs230 can efficiently block transmission. However, little is known about the epitope specificity and potency of monoclonal antibodies (mAbs) that make up the functional polyclonal response. Here, we (i) identified and characterized human mAbs against Pfs48/45 and define new protective epitopes, and (ii) provide new insights into the affinity, potency and epitope specificity of mAbs targeting Pfs230. Peripheral blood mononuclear cells were selected from expatriates living in Central Africa and Ugandans with serum reactivity against Pfs48/45 and Pfs230, and transmission-reducing activity (>99%) of total IgG in standard membrane feeding assays (SMFAs). Two approaches were followed to identify reactive B cells: i) single B cell sorting with labeled Pfs48/45 and ii) a microfluidic single-cell assay to identify B cells producing antibodies against Pfs48/45 or Pfs230. IgG genes from single cells were sequenced and cloned mAbs were produced in eukaryotic cells. Binding validation was done by flow cytometry and native protein recognition was confirmed by surface-immunofluorescence assay using gametes. Affinity was measured by surface plasmon resonance (SPR) and transmission-reducing potency is currently being assessed in SMFA. So far, we have confirmed transmission-reducing activity when testing Pfs48/45-specific mAbs (>85% reduction). Binding and functional data of approximately 100 unique Pfs48/45- and Pfs230-reactive mAbs will be presented. These novel transmission-blocking mAbs from naturally exposed donors against these two promising targets provide new insights for vaccine development.

1610

LILRB1 AND LILRB2 EXPRESSION IN PERIPHERAL BLOOD IMMUNE CELLS AT 18 AND 24 MONTHS OF AGE IN INFANTS BORN FROM MOTHERS WITH PLACENTAL MALARIA

Celia Dechavanne[1], Odilon Nouatin[2], Rafiou Adamou[3], Sofie M. Edslev[4], Anita Hansen[5], Ibrahim Sadissou[3], Erasme Gbaguidi[3], Jacqueline Milet[1], Gilles Cottrell[1], Laure Gineau[1], Audrey Sabbagh[6], Achille Massougbodji[7], Kabirou Moutairou[2], Eduardo A. Donadi[8], Edgardo D. Carosella[9], Philippe Moreau[9], Edmond J. Remarque[10], Michael Theisen[11], Nathalie Rouas-Freiss[9], Andre Garcia[1], Benoit Favier[12], David Courtin[1]

[1]*UMR261 - MERIT - Institut de Recherche pour le Developpement (IRD), Paris, France,* [2]*Faculté des Sciences et Techniques (FAST), Cotonou, Benin,* [3]*UMR261 - MERIT - Centre d'Étude et de Recherche sur le Paludisme Associé à la Grossesse et l'Enfance (CERPAGE), Cotonou, Benin,* [4]*Department of Bacteria, Parasites, and Fungi - Staten Serum Institute, Copenhagen, Denmark,* [5]*Staten Serum Institute, Copenhagen, Denmark,* [6]*UMR261 - MERIT - Université de Paris, Paris, France,* [7]*Centre d'Étude et de Recherche sur le Paludisme Associé à la Grossesse et l'Enfance (CERPAGE), Cotonou, Benin,* [8]*Division of Clinical Immunology, Department of Medicine, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil,* [9]*Commissariat à L'Energie Atomique Et Aux Energies Alternatives (CEA), Direction de La Recherche Fondamentale (DRF), Service de Recherche en Hémato-Immunologie (SRHI), Paris, France,* [10]*Department of Parasitology - Biomedical Primate Research Centre, Rijswijk, Netherlands,* [11]*Department for Congenital Disorders, Statens Serum Institut, Copenhagen, Denmark,* [12]*U1184 - IDMIT Department - Commissariat à L'Energie Atomique Et Aux Energies Alternatives (CEA), Direction de La Recherche Fondamentale (DRF), Fontenay-aux-Roses, France*

Placental malaria (PM) was associated with a higher susceptibility of infants to malaria. A hypothesis of immune tolerance was suggested but no clear explanation has been provided so far. In this work, we were interested in the inhibitory receptors LILRB1 and LILRB2 that are expressed at the surface of immune cells and play an essential role in the modulation of their functions such as cell differentiation, proliferation and cytotoxicity. Therefore, in this study, we hypothesize that LILRB1 and LILRB2 expression could be involved in PM-induced immune tolerance. In Benin, West Africa, 345 newborns were enrolled and followed-up to 24 months of age. Data of specific IgG1, IgG2, IgG3 and IgM for seven blood stage antigens, immune cell phenotyping (T cells, B cells, NK cells, γδT cells, monocytes, neutrophils and eosinophils) as well as LILRB1 and LILRB2 expression were collected at 18 and 24 months of age for 154 infants. Compared to infants born from mothers without PM, we have found that infants born from PM-mothers had a higher risk of developing clinical malaria (IRR=1.53 [1.02;2.31], p=0.040), a higher frequency of classical monocytes (OR=1.15 [1.03;1.28], p=0.01) and a lower frequency of non-classical monocytes (OR=0.74 [0.59;0.94], p=0.01) with a higher LILRB2 expression at their surface (OR=1.36 [1.12;1.66], p>0.01) between 18 and 24 months of age. Moreover, infants born from PM-mothers developed less IgG1 and IgG3 including IgG1 to MSP2-FC27 (OR=0.45 [0.30;0.69], p>0,001) and IgG1 to MSP3 (OR=0.79 [0.67;0.93], p=0.006). First, our results suggest that infants born from PM-mothers develop a regulated non-classical monocyte response at 18 and 24 months of age. Second, the same infants developed lower IgG levels suggesting an impaired capability of neutralizing the pathogen. Taken together, we speculate that less IgGs could also impair monocytes function such as opsonization and phagocytosis in infants born from PM-mothers, which could contribute to their higher susceptibility to malaria.

1611

CD8⁺ T CELLS: A PARADIGM SHIFT IN TREATING CEREBRAL MALARIA?

Brittany A. Riggle, Louis H. Miller, Susan K. Pierce

National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

Mosquito-transmitted *Plasmodium falciparum* infection causes malaria and is disproportionately fatal in young children in Africa. Malaria-associated fatalities are overwhelmingly caused by cerebral malaria, a complication that does not adequately respond to intravenous antimalarial therapy. The abundance of infected red blood cells that accumulate in the cerebral vasculature of patients has led to the belief that these brain-sequestered infected red blood cells were solely responsible for severe disease. However, mouse models of cerebral malaria have implicated $CD8^+$ T cells in pathogenesis, but lack of human corroboration has slowed efforts to identify therapeutic targets. We performed multiplex immunohistochemistry in post-mortem brain samples from children with or without cerebral malaria and with known HIV status. Cerebral malaria diagnosis was associated with elevated numbers of $CD3^+CD8^+$ T cells engaging the cerebrovasculature. HIV co-infection further increases $CD3^+CD8^+$ T cell engagement as well as enhanced distribution into the cerebral perivasculature. These data provide a rationale for investigating $CD3^+CD8^+$ T cells as the target of an adjunctive therapy for cerebral malaria.

1612

CD163 GENE EXPRESSION AND SOLUBLE CD163 LEVELS INCREASE IN MALARIA INFECTED PREGNANT WOMEN

Bartholomew N. Ondigo[1], Ian N. Moore[2], Sundar Ganesan[3], Kevin W. Bock[2], Paul S. Blank[4], Almahamoudou Mahamar[5], Oumar Attaher[5], Bacary S. Diarra[5], Youssoufa Sidibe[5], Jillian Neal[6], Alassane Dicko[5], Patrick E. Duffy[6], Michal Fried[6]

[1]*Department of Biochemistry and Molecular Biology, Egerton University - Kenya, Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States,* [2]*Comparative Medicine Branch, Infectious Disease Pathogenesis Section, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States,* [3]*Research Technologies Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States,* [4]*Section on Integrative Biophysics, Division of Basic and Translational Biophysics, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, United States,* [5]*Malaria Research & Training Center, Faculty of Medicine, Pharmacy and Dentistry, University of Sciences Techniques and Technologies of Bamako, Bamako, Mali,* [6]*Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States*

During placental malaria (PM), *Plasmodium falciparum*-infected red blood cells sequester in the placenta, resulting in accumulation of maternal macrophages in the intervillous spaces. Previously, we reported that PM was associated with increased levels of proinflammatory cytokines TNF-α and IFN-γ, the chemokine CXCL9, and IL-10. In this study, we characterize the PM inflammatory response by profiling maternal macrophages in intervillous spaces and fetal Hofbauer cells (HBC) in placenta samples collected from women found to be infected during pregnancy or at delivery. We conducted an immunohistochemistry analysis of placental tissue samples using M1 and M2 surface markers, quantified surface marker transcripts using qRT-PCR, and measured soluble CD163 (sCD163) levels in primigravid maternal blood collected during pregnancy and at delivery. Relative gene expression of CD163 was measured in 30 placental samples (PM-, n=10; PM+ during pregnancy, n=9; PM+ during pregnancy and at delivery, n=11), and was significantly higher in women with at least one infection during pregnancy compared to uninfected women (p=0.02). sCD163 levels (resulting from shedding surface CD163) were

measured in 34 peripheral blood samples collected at gestational week 30-32 (PM-, n=12; PM+ at sampling or before, n=22) and in 40 placental blood samples (PM-, n=13; PM+ during pregnancy, n=14; PM+ during pregnancy and at delivery, n=13) from the same primigravid mothers. Compared to uninfected women, sCD163 levels were significantly elevated in women infected at gestational week 30-32 (p=0.008) and at delivery or before (p=0.003). Further, placental blood sCD163 levels correlated with the number of infections (r=0.54, p=0.001). Increased sCD163 in PM+ women suggest activation of M2 macrophages and may explain increased IL-10 levels especially during chronic placental malaria. M1 and M2 macrophages in placental intervillous spaces and HBC are currently being quantified by immunohistochemistry and these results will be presented. This study will further provide insight into the contribution of maternal macrophages (M1 and M2) and HBC in PM pathogenesis.

1613

REPEATED CONTROLLED HUMAN MALARIA INFECTION IN AFRICAN ADULTS TO DISSECT NATURALLY-ACQUIRED IMMUNITY

Matthew B. McCall[1], F. Jeannot Zinsou[1], B. Roméo Adégbitè[1], Rafiou Adamou[1], Hutch H. Nzenguele[1], Paulin N. Essone[1], Yonas Abebe[2], B. Kim Lee Sim[2], Bertrand Lell[1], Sélidji T. Agnandji[1], L.W. Preston Church[2], Thomas L. Richie[2], Ayola A. Adegnika[1], Stephen L. Hoffman[2], Peter G. Kremsner[3], Benjamin Mordmüller[3]

[1]*Centre de Recherches Médicales de Lambaréné (CERMEL), Lambaréné, Gabon,* [2]*Sanaria Inc., Rockville, MD, United States,* [3]*Institut für Tropenmedizin (ITM), Tübingen, Germany*

Development of naturally acquired immunity (NAI) to malaria is poorly understood. Controlled human malaria infections (CHMIs), whereby volunteers are purposefully inoculated with *Plasmodium falciparum* (Pf) sporozoites, are powerful tools to dissect NAI, and evaluate malaria vaccines. Here we evaluate the course of repeated CHMIs in healthy, lifelong malaria-exposed Gabonese adults, using aseptic, purified, cryopreserved Pf sporozoites (PfSPZ Challenge) (PfNF54, African origin) and PfSPZ Challenge (Pf7G8, Brazilian origin). Pf7G8 differs more from PfNF54 at the genomic level than 700 other African Pf isolates differ from PfNF54. Fifty-six volunteers were randomised to one of two sequences involving one Pf7G8 and five PfNF54 infections at 8-week intervals. Subjects were inoculated by direct venous inoculation with $3.2x10^3$ PfSPZ (a dose fully infective to malaria-naïves) and followed by thick blood smear (TBS). Subjects developing parasitaemia with symptoms were treated (under supervision) with artemether-lumefantrine (AL). To minimise risk of onward transmission of non-African parasites, 7G8 infections were treated with AL as soon as TBS positive (regardless of symptoms), or else presumptively at day 17 post-inoculation, plus a single dose of primaquine to kill gametocytes. Follow-up is complete for 99 individual CHMIs (29 with Pf7G8 and 70 with PfNF54), with ~160 more CHMIs scheduled over the next 6 months. Inoculations were safe and well-tolerated, with no related grade 3 or serious adverse events. The proportion of subjects developing patent parasitaemia by TBS within 17 days following inoculation was 12/29 (41%) for Pf7G8 and 34/70 (49%) for PfNF54. Strikingly, all 12 subjects with Pf7G8 parasitaemia developed malarial symptoms, but only 3/34 (9%) of subjects with PfNF54 parasitaemia prior to day 17 had symptoms. Median parasitaemia in subjects with malarial symptoms was nonetheless ~10-fold lower for Pf7G8 than for PfNF54. The data demonstrate divergent parasitological and clinical responses to Pf7G8 and PfNF54, expand our understanding of NAI to malaria and aid in rational design of live-attenuated malaria vaccines.

1614

SINGLE AND REPEATED PRAZIQUANTEL TREATMENTS SIGNIFICANTLY REDUCE *SCHISTOMA* INFECTION INTENSITY BUT SHOW A POOR CURE RATE AS DEMONSTRATED BY URINE CIRCULATING ANODIC ANTIGEN DIAGNOSTICS: RESULTS FROM THE REPST TRIAL

Pytsje T. Hoekstra[1], Miriam Casacuberta-Partal[1], Rufin K. Assaré[2], Kigbafori D. Silué[2], Roula Tsonaka[3], Evelien van Kaathoven[1], Claudia J. de Dood[4], Paul L. Corstjens[4], Stefanie Knopp[5], Juerg Utzinger[5], Jean T. Coulibaly[2], Lisette van Lieshout[1], Govert J. van Dam[1]

[1]*Department of Parasitology, Leiden University Medical Center, Leiden, Netherlands,* [2]*Centre Suisse de Recheres Scientifiques en Côte d'Ivoire, Abidjan, Côte D'Ivoire,* [3]*Department of Biomedical Data Sciences, Leiden University Medical Center, Leiden, Netherlands,* [4]*Department of Cell and Chemical Biology, Leiden University Medical Center, Leiden, Netherlands,* [5]*Swiss Tropical and Public Health Institute, Basel, Switzerland*

Preventive chemotherapy with praziquantel (PZQ) is the cornerstone of schistosomiasis control. So far, most studies assessing PZQ efficacy have used relatively insensitive parasitological diagnostic methods, thereby overestimating cure rates (CR) and intensity reduction rates (IRR). To determine the efficacy of PZQ more accurately, we employed the ultra-sensitive and highly specific UpConverting Phosphor labelled, Lateral Flow (UCP-LF) assay for the detection of Circulating Anodic Antigen (CAA). An open-label, randomized controlled trial (NCT02868385) was conducted in Taabo, Côte d'Ivoire. School-aged children with a confirmed *S. mansoni* infection, based on Kato-Katz (KK) and the Point-of-Care Circulating Cathodic Antigen (POC-CCA) urine test, were randomly assigned to receive either a single dose of PZQ or four repeated doses at two-week intervals (Hoekstra *et al*., 2020). Here, we present the outcome of the urine UCP-LF CAA assay in terms of CR and IRR for both groups, measured 10 weeks after the first treatment. During baseline screening 1,022 children were assessed for eligibility of whom 153 (15%) had a detectable *S. mansoni* infection, and hence, were randomized to a standard treatment group (N=70) and an intense treatment group (N=83). In both groups, a substantial reduction in urine CAA levels was observed, with an IRR of 89% and 94% in the standard and intense treatment group, respectively. However, a CR of only 19% and 23% was observed in the standard and intense treatment group, respectively. By employing the more sensitive and genus specific UCP-LF CAA diagnostic test, the observed CR confirms the already published poor CR in this study population based on POC-CCA. Even though a significant reduction in intensity of infection was observed, the total number of CAA-positive individuals did not decrease significantly despite repeated treatment, indicating that active Schistosoma infections were still present in our study population. Our findings stress the need for reliable and more sensitive diagnostic tools especially to accurately monitor the effect of PZQ and to optimize treatment protocols.

1616

COMPARISON OF POC-CCA WITH KATO-KATZ IN DIAGNOSING *SCHISTOSOMIASIS MANSONI* INFECTION IN A PEDIATRIC L-PRAZIQUANTEL CLINICAL TRIAL

Xiaoyan Yin[1], Brooke Hayward[1], Wilhelmina Bagchus[2], Oezkan Yalkinoglu[2], Deon Bezuidenhout[2], Elly Kourany-Lefoll[2]

[1]*EMD Serono, Billerica, MA, United States,* [2]*Merck Healthcare KGaA, Darmstadt, Germany*

Traditionally *Schistosomiasis mansoni* infection is diagnosed by Kato-Katz (KK) method: multiple stool samples of a person are collected over 3 to 5 days. Smears of each sample are read under a microscope for egg counts. Point-of-care circulating cathodic antigen (POC-CCA) cassette, a commercially available tool, detects schistosomiasis antigens from urine samples in 20 minutes. POC-CCA results can be negative, positive 1+, 2+ or 3+, with more + indicating more worm antigens in the sample. Both methods were used in a phase II trial investigating the efficacy and safety of pediatric L-Praziquantel (PZQ) orally disintegrating tablets among

children ≤ 6 years, and the consistency between the two methods was evaluated. POC-CCA was used to prescreen for *S. mansoni* infection. Children with positive results were tested again by KK, and those with positive KK results (>1 egg) were enrolled. Participants (n=444) were treated with different formulations and/or doses of PZQ. POC-CCA and KK were performed at 2-3 weeks after treatment to evaluate drug efficacy. Cure rate (CR) was defined as the proportion of participants with negative result per POC-CCA or no eggs in stool sample per KK. Kappa statistic was calculated to assess the agreement on cure status, and Spearman correlation between POC-CCA positiveness and KK egg counts was calculated. Sensitivity and specificity of POC-CCA were evaluated using KK as a reference standard, though with reservations that KK is not perfectly sensitive especially among young children. CR per POC-CCA reached 52% with 95%CI [48%, 57%] at 2-3 weeks after treatment across all treatment groups. CR per KK was higher, 83% with 95%CI [79%, 87%]. Kappa statistic was 0.16 with 95%CI [0.09, 0.23], indicating that the agreement was slightly better than just by chance. Relative to KK, POC-CCA's sensitivity to detect infection was 70% and specificity for cure was 57%. Spearman correlation coefficient between POC-CCA positivity and KK egg counts was 0.26 ($p<0.0001$). POC-CCA is sensitive and rapid for diagnosing S. *mansoni* infection, but its performance and consistency with KK need to be further validated especially in young children.

1617

EVALUATION OF REPORTER PARTICLES TO DEVELOP A POINT-OF-CARE LATERAL FLOW ASSAY TO DIAGNOSE *SCHISTOSOMA* INFECTION

Elías Kabbas-Piñango[1], Arnaud Chalin[2], Paul L. Corstjens[3], Maxime Laroche[2], Milovan Stankov[2], Govert J. van Dam[3], Jonathan M. Cooper[1], Poppy H. Lamberton[1]

[1]*University of Glasgow, Glasgow, United Kingdom,* [2]*NG Biotech, Guipry-Messac, France,* [3]*Leiden University Medical Center, Leiden, Netherlands*

Traditional microscopy diagnostic methods for schistosomiasis are specific but lack sensitivity. More sensitive methods are available, however many require laboratory resources. A Circulating Cathodic Antigen (CCA) Lateral Flow Immunoassay (LFIA) is mainly suited for *Schistosoma mansoni* infections. The Circulating Anodic Antigen (CAA) is a genus-specific target for all *Schistosoma* species. A sensitive laboratory-based LFIA uses Up-Converting Phosphor (UCP) reporter particles for the determination of CAA levels in urine and serum, although pre-treatment, centrifugation and sample incubation are needed. The UCP-LF CAA test can detect down to 10 pg/mL utilising 10-20 µL of acid extracted urine or serum but takes approximately 2 hours. Here we evaluate different nanoparticles to detect CAA without preincubation in a rapid Point-Of-Care (POC) LF test format. Different nanoparticles (colloidal gold, magnetic particles, europium and UCP) were conjugated to a monoclonal antibody anti-CAA and assembled on a conjugate pad, with a CAA-specific capture line on the LF strip using the same antibody. Purified CAA was spiked into 100 µL of buffer and CAA-negative urine samples for the determination of sensitivity and specificity. Colloidal gold and magnetic particles showed a sensitivity of 500-1000 pg/mL and allowed a naked-eye qualitative reading of the test result. Europium and UCP particles required a reader. The europium LFIA did not detect CAA levels below 1000 pg/mL, whilst the UCP devices detected CAA at 200 pg/mL which would allow detection of moderate to high intensity infections. All reporter particles showed high specificity. These results suggest UCP particles are the most sensitive for the detection of CAA, although further optimisation of the LFIA is needed to reach higher sensitivity and meet the necessary POC standards. A sensitive, easy to perform and analyse POC-CAA LFIA would enable the diagnosis of all types of human schistosomiasis including low intensity infections, thus contributing to improved drug efficacy monitoring and surveillance in endemic areas in order to help control and eliminate this debilitating disease.

1619

DISCOVERY AND DEVELOPMENT OF HIGHLY POTENT AND EFFICACIOUS IMIDAZOPYRAZINE DERIVATIVES FOR THE TREATMENT AND PREVENTION OF SCHISTOSOMIASIS

Shashank Kulkarni[1,5], Mark Gardner[2], Nuha Mansour[3], Andy Bell[2], Quentin Bickle[2], Axel Becker[4], Philip Hewitt[4], Nico Alexander Mell[4], Lassina Badolo[4], Thomas Spangenberg[5]

[1]EMD Serono R&D Institute, Billerica, MA, USA[2]Salvensis, Sandwich, Kent, CT13 9ND, UK[3]The London School of Hygiene & Tropical Medicine, London WC1E 7HT, UK[4]Merck Healthcare KGaA, Darmstadt, Germany[5]Global Health Institute of Merck, Ares Trading S.A., a subsidiary of Merck KGaA (Darmstadt, Germany), Eysins, Switzerland

Schistosomiasis is a chronic parasitic disease caused by the trematode flatworms of the genus Schistosoma. It is a life-threatening neglected tropical disease that affects more than 250 million people worldwide. Human schistosomiasis is considered one of the most devastating parasitic disease, second only to malaria. Praziquantel still remains the only drug on the market that is active against the adult stages of three major species of Schistosoma. Our goal at the Global Health Institute is to develop transformative health solutions to gain control of schistosomiasis-induced morbidities, to prevent and to eliminate this disease as a public health burden. With the emerging threat of resistance to Praziquantel, it is important to identify and develop new drug candidates. A novel, potent and fast acting chemotype discovered in a collaboration between LSHTM and Salvensis shows efficacy against juvenile and adult worms. This series meets all the attributes of the desired Target Product Profile in the field of Schistosomiasis for treatment and prevention. The lead candidate has a low projected human dose, high activity on juvenile and adult worms along with good bioavailability and attractive pharmacodynamic profile.

1620

USE OF A TABLET-BASED SYSTEM WITH PORTABLE TRANSDUCERS TO PERFORM ABDOMINAL ULTRASOUNDS IN A FIELD INVESTIGATION OF SCHISTOSOMIASIS-RELATED MORBIDITY

Anne Straily[1], Alfred Malit[2], Dollycate Wanja Njagi[2], MORBID Ultrasound Team Kenya --[3], Emmy Awino Kavere[2], Ryan E. Wiegand[1], Susan P. Montgomery[1], Alie Eleveld[2], Alex Mwaki[2], William E. Secor[1], Maurice R. Odiere[4]

[1]*Centers for Disease Control and Prevention (CDC), Atlanta, GA, United States,* [2]*Safe Water and AIDS Project, Kisumu, Kenya,* [3]*--, Kisumu, Kenya,* [4]*Safe Water and AIDS Project, Kenya Medical Research Institute, Kisumu, Kenya*

The goal of schistosomiasis control programs is to reduce morbidity but methods to directly measure morbidity are lacking. Success is instead evaluated using reductions in infection prevalence or intensity. The Morbidity Operational Research for Bilharziasis Implementation Decisions (MORBID) study was designed to determine alternative approaches to evaluate schistosomiasis-related morbidity. Ultrasound can be used to directly visualize organomegaly, is non-invasive, and can distinguish *Schistosoma mansoni*-related liver fibrosis from cirrhosis. However, ultrasound historically has not been feasible for routine, field-deployable use in schistosomiasis control programs because of cost, logistic limitations, and lack of experienced sonographers. The MORBID study for intestinal schistosomiasis was piloted in an area of western Kenya with high endemicity of *S. mansoni* infections. Abdominal ultrasounds were performed using a tablet-based system, whereby the transducer is connected to a compatible smart device. Sonographers were recent graduates of medical imaging programs and given additional training in identifying schistosomiasis-specific pathology according to the WHO Niamey protocol. In total, 6,477 participants were examined over four months; scans required about 20 minutes per participant. In addition to hepatosplenic schistosomiasis, other pathologies were identified, such

as uterine fibroids, polycystic kidney disease, and hydronephrosis; these participants were referred for additional care. Pregnancies were also diagnosed among several women who were unaware they were pregnant. Using a tablet-based ultrasound system resolved previous challenges to conducting ultrasound in the field: it was light, easily transportable, did not require an external power source, was more affordable than a standard ultrasound machine, and high-resolution images could be saved directly to the device. Such a system may make it more feasible to implement abdominal ultrasounds in schistosomiasis control programs and assess liver and spleen morbidity, while also offering significant ancillary benefits to the community.

1621

EFFECT OF DRINKING WATER CHLORINATION ON FECAL CARRIAGE OF CULTURABLE ANTIMICROBIAL RESISTANT BACTERIA IN BANGLADESHI CHILDREN: RESULTS FROM A RANDOMIZED CONTROLLED TRIAL

Maria Camila Montealegre[1], Esther E. Greenwood[1], Lisa Teichmann[1], Maya L. Nadimpalli[2], Lea Caduff[1], Jenna Swarthout[2], Tabea Nydegger[1], Mohammad A. Islam[3], Val F. Lanza[4], Stephen P. Luby[5], Amy J. Pickering[2], Timothy R. Julian[1]

[1]*Eawag, Duebendorf, Switzerland,* [2]*Tufts University, Medford, MA, United States,* [3]*Washington State University, Pullman, WA, United States,* [4]*Hospital Universitario Ramón y Cajal, Madrid, Spain,* [5]*Stanford University, Stanford, CA, United States*

Water, sanitation and hygiene (WASH) services have the potential to interrupt transmission of antimicrobial resistant (AMR) bacteria and reduce antibiotic use, thereby reducing selective pressure. However, evidence on the efficacy of WASH to combat AMR is lacking. Here, we evaluated the presence and concentration of culturable extended-spectrum β-lactamase (ESBL)-*Escherichia coli* and ESBL-KESC (*Klebsiella* spp., *Enterobacter* spp., *Serratia,* and *Citrobacter* spp.) in the feces of 479 Bangladeshi children <5 years of age enrolled in a double-blind, randomized controlled trial of in-line drinking water chlorination. The trial, conducted in 2015 in two low income urban communities in Bangladesh, demonstrated significant reductions in diarrheal disease. We detected ESBL-*E. coli* and ESBL-KESC in the feces of 64.5% (n=309) and 11.7% (n=56) of children, respectively. We observed no statistically significant difference in the prevalence of ESBL-*E. coli* (generalized linear model (glm), estimate= 0.024 [Group B], p= 0.83) or ESBL-KESC (glm, estimate= 0.27 [Group B], p= 0.33) among children in the treatment group compared to the control, when controlling for study site. ESBL-*E. coli* concentrations were not significantly different, with mean (standard deviation) of 4.14 (1.49) in Group A and 3.96 (1.35) in Group B expressed as $\log_{10}$ CFU/g-wet feces (linear model (lm), estimate= -0.13[Group B], p= 0.38, r^2= 0.01). Similarly, ESBL-KESC concentrations, 2.93 (1.08) in Group A compared to 3.51 (1.30) in Group B were not significantly different (lm, estimate = 0.10 [Group B], p = 0.09, r^2=0.004). Prevalence and concentration of ESBL-*E. coli* differed by study site, but not KESC. Overall, the findings highlight that in-line drinking water chlorination effective at reducing diarrheal disease is insufficient alone to meaningfully impact carriage of ESBLs in an area of high disease transmission. Development and evaluation of effective strategies to control carriage of AMR are needed to support National and Global Action Plans calling for improved WASH services.

1622

IMPACT OF DRINKING WATER CHLORINATION ON CHILDREN'S GUT MICROBIOMES IN DHAKA, BANGLADESH

Maya L. Nadimpalli[1], Val F. Lanza[2], Maria Camila Montealegre[3], Lisa Teichmann[3], Lea Caduff[3], Jenna Swarthout[1], Sonia Sultana[4], Mohammad Aminul Islam[5], Stephen P. Luby[6], Timothy R. Julian[3], Amy J. Pickering[1]

[1]*Tufts University, Medford, MA, United States,* [2]*Hospital Universitario Ramón y Cajal, Madrid, Spain,* [3]*Eawag, Dübendorf, Switzerland,* [4]*International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh,* [5]*Washington State University, Pullman, WA, United States,* [6]*Stanford University, Stanford, CA, United States*

Healthy assembly of the gut microbiome provides long-term benefits. Bangladeshi children are often exposed to diarrheal pathogens and antibiotics, which perturb normal microbiome development. A cluster randomized controlled trial in two low-income communities in Dhaka found that point-of-collection drinking water chlorination reduced diarrheal disease by 23% and recent antibiotic consumption by 7% among children under five. We leveraged stool samples collected one year after initiation of the intervention to examine whether drinking water chlorination also impacted children's gut microbiota, including the antibiotic resistance genes (ARGs) they harbored. We performed metagenomic short-read sequencing on fecal DNA from 95 randomly selected treatment and control children aged 6-61 months. We classified the taxonomy of raw reads and identified ARGs using ResFinder. We examined group differences in genera richness and relative abundance of genera and ARGs while controlling for age and study site. Groups will be unblinded when analysis is complete. The impacts of drinking water chlorination on children's gut microbiota differed by age. Specifically, children in Group B whose gut microbiomes were likely developing (age <31 months, n =16) had at least 2-fold higher abundance of genera containing known pathogens (*Shigella* spp. and *Helicobacter* spp.) than similarly aged Group A children (n=17). Their microbiomes were also more likely to harbor the $bla_{\text{CTX-M-15}}$ ESBL gene, which confers 3rd generation cephalosporin resistance, than Group A children. Meanwhile, children in Group B over ≥ 31 months (n=33) had lower abundance of *Akkermansia* spp., which are associated with reduced inflammation, than Group A children (n=29). Drinking water chlorination increased the taxonomic richness of younger but not older children's gut microbiota. Overall, a cluster randomized drinking water treatment intervention that reduced children's diarrhea and antibiotic consumption in urban Bangladesh also impacted the composition of children's gut microbiomes and whether they harbored the $bla_{\text{CTX-M-15}}$ ESBL gene, but impacts differed by age.

1623

IDENTIFICATION OF MULTI-DRUG RESISTANT BACTERIA IN PRIMARY DRINKING WATER SOURCES IN SOUTHWEST COASTAL BANGLADESH

Sarker Masud Parvez[1], Davidson H. Hamer[2], Jean M. van Seventer[3], Mohammad Aminul Islam[4], Mohammad Badrul Amin[1], Nafisa Halim[5], Jeffrey K. Griffiths[6], Tremearne Hotz[5], Mahbubur Rahman[1]

[1]*International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh,* [2]*Section of Infectious Diseases, Department of Medicine, Boston University School of Medicine, Boston, MA, United States,* [3]*Department of Environmental Health, Boston University School of Public Health, Boston, MA, United States,* [4]*Paul G. Allen School for Global Animal Health, Washington State University, Pullman, WA, United States,* [5]*Department of Global Health, Boston University School of Public Health, Boston, MA, United States,* [6]*Department of Public Health & Community Medicine, Tufts University, Boston, MA, United States*

Ponds in southwest coastal Bangladesh are potentially contaminated with antibiotics and multi-drug resistant (MDR) bacteria from feces of humans and animals in the surrounding environment. Due to high ground water salinity during the dry season, local inhabitants use ponds water for their primary drinking source. We hypothesized that, household members who use pond water for drinking are exposed to MDR bacteria. To test this hypothesis, we sampled water from 20 ponds (≥150 m^2) which serve as a drinking water source for ≥20 households and have commercial shrimp aquaculture operations and/or livestock/poultry husbandry operations in the immediate vicinity. *Escherichia coli* and *Enterococcus spp.* isolates from each water sample were analyzed for resistance to clinically important antibiotics using the disk diffusion method. We also randomly surveyed 20 households within 500 meters of each pond (total 400 households). During the dry season, nearly all (96%) respondents drank pond water

while the average reported home water storage duration was 8.5 days. More than 90% of respondents reported recently treating pond water with aluminum potassium sulfate (alum) before drinking. Only 1% and 4% of respondents reported recently boiling or filtering water, respectively. About 85% of household's raise animals and 45% of their animals have direct contact with drinking water pond. For each pond, at least one of four bacterial isolates (2 *E. coli* & 2 *Enterococcus spp.)* was resistant to at least one antibiotic. *E. coli* isolates were predominantly resistant to ampicillin (n: 10, 50%) followed by ceftriaxone/cefotaxime/cefixime (n: 8, 40%); sulfamethoxazole/ trimethoprim (n: 7, 35%); cefepime (n: 6, 30%); nalidixic acid (n: 3, 15%); and ciprofloxacin (n: 2, 10%). *Enterococcus* isolates. showed resistance to ampicillin (n: 1, 5%), vancomycin (n: 2, 10%) and linezolid (n: 2, 10%). These results imply that, households in southwest coastal Bangladesh are at risk of exposure to MDR bacteria through the use of ponds as drinking water sources during the dry season which may have adverse health consequences.

1624

ANALYSIS OF HOUSEHOLD ANTIBIOTIC USE AND MULTIDRUG RESISTANT BACTERIAL CONTAMINATION OF DRINKING WATER PONDS IN SOUTHWEST COASTAL BANGLADESH

Jean Maguire van Seventer[1], Tremearne Hotz[1], Sarker M. Parvez[2], Emily E. van Seventer[1], Mohammad A. Islam[3], Mohammed B. Amin[2], Nafisa Halim[1], Jeffrey K. Griffiths[4], Davidson H. Hamer[1], Mahbubur Rahman[2]

[1]*Boston University School of Public Health, Boston, MA, United States,* [2]*International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh,* [3]*Paul G. Allen School for Global Animal Health, Washington State University, Washington, MA, United States,* [4]*Tufts University School of Medicine, Boston, MA, United States*

Antimicrobial resistance (MDR), a threat to global health, must be addressed at the human-animal-environment interface using a One Health approach. In southwest coastal Bangladesh, climate change drives rural communities to abandon high salinity well water in favor of low salinity pond water for household use. We hypothesize that humans are exposed to multidrug resistant (MDR) bacteria because of consuming inadequately treated water from ponds receiving rainy season surface runoff contaminated with human and animal feces containing antibiotics and MDR bacteria. To test this hypothesis, we collected data on water quality from 20 community drinking water ponds (>150 m^2) in two locations (Dacope, n=10; Bagerhat, n=10), and human and animal antibiotic usage by 20 households within 500 meters of each pond (n=400). There was a trend toward *Escherichia coli* and/or *Enterococcus* spp. in Dacope ponds having a higher number of isolates resistant (R) to certain antibiotics than in Bagerhat ponds (*E. coli* R to ampicillin (p= 0.07), ceftriaxone (p=0.17), and/or sulfamethoxazole/trimethoprim (p=0.35), and *Enterococcus* spp. R to erythromycin (p=0.37). The median distance to the nearest river was significantly closer (p<0.0001) for Dacope ponds than Bagerhat ponds (median distance 376 vs. 5953 meters). Ponds with closer proximity to rivers were associated with *E. coli* R to ampicillin (p= 0.01), ceftriaxone (p=0.01), and/or sulfamethoxazole/trimethoprim (p=0.16), and *Enterococcus* spp. R to erythromycin (p=0.71). Ponds with more households using penicillins, cephaloporins, quinolones/fluoroquinolones, and/or sulfonamides and/or trimethoprim were at higher odds of being from Dacope ponds (OR, respectively, 1.45; 1.96; 6.38; 1.05). Household drinking water treatment was inadequate to remove bacteria (data not shown). Although a pilot study of small sample size, these data suggest that both environmental factors (drinking water pond location) and behavioral factors (antibiotic usage and drinking water treatment) contribute to human exposure to MDR bacteria.

1625

THE ASSOCIATION BETWEEN CLIMATE AND SAFE DRINKING WATER USE: A MULTI-COUNTRY ANALYSIS

Andrea Geri Buchwald[1], Anna Roose[2], Kristopher B. Karnauskas[1], Karen L. Kotloff[2], Elizabeth J. Carlton[1]

[1]*Colorado School of Public Health, Aurora, CO, United States,* [2]*Center for Vaccine Development and Global Health, Baltimore, MD, United States*

Climate change may alter access to safe drinking water, with important implications for health, including diarrheal diseases. We utilized data from a case-control study of diarrhea to assess the relationship between temperature and rainfall and utilization of safe drinking water in Gambia, Mozambique, Pakistan, and Kenya. Demographics, wealth indicators, drinking water sources, the time required to collect water, and frequency of availability were collected. The primary outcome of interest was whether the reported main drinking water source used in the past two weeks met the definition of "Basic Safe Drinking Water" (BSDW) by the World Health Organization, and a secondary outcome was use of a BSDW source that was always available. Temperature and precipitation data were compiled from local weather stations and the Climate Hazards Group InfraRed Precipitation with Station data, respectively, and summarized to account for long and short-term weather patterns as well as potential lags. Machine learning was used to identify the most important weather variables in predicting the outcome for each site and logistic regression was used to identify the direction and magnitude of association between the most important weather variables and BSDW source use. Increasing household wealth was the most important predictor of BSDW use across all sites. Increasing rainfall, both in the long and short term, was associated with increased use of BSDW sources in Mozambique and Kenya. High temperatures were associated with decreased use of BSDW in Mozambique, Kenya, and Pakistan. For the secondary outcome, increasing precipitation was associated with increased use of BSDW that was always available in Kenya, however, in contrast to the primary outcome, increasing precipitation was associated with decreased use of BSDW that was always available in Gambia and Mozambique. High temperature, on both a long and short scale, was associated with decreased use of BSDW that was always available at all study sites. While mechanisms by which climate alters safe water use and access vary by location, climate change may exacerbate these effects in all low-resource settings.

1626

KEEPING WATER SAFE IN HUMANITARIAN CRISES: AN EXPLORATORY STUDY ON FACTORS INFLUENCING CHLORINE DECAY IN REFUGEE CAMPS IN SOUTH SUDAN, JORDAN, AND RWANDA

Syed Imran Ali

Dahdaleh Institute for Global Health Research, York University, Toronto, ON, Canada

Waterborne diseases are among the leading threats facing displaced populations during humanitarian crises, especially in refugee and internally displaced persons (IDP) camps. In these settings, safe water is essential for protecting public health. Water chlorination is the most widely used method of water treatment in emergencies because it provides residual protection from recontamination by waterborne pathogens. In refugee/IDP camp settings, water-users must collect water from public distribution points and transport it to their shelters, where they store and use it for up to 24 hours or longer. Environmental hygiene conditions are often poor in refugee/IDP camp settings, creating many opportunities for waterborne pathogens and other organic contaminants to consume residual chlorine protection in treated water leaving it vulnerable to pathogenic recontamination. Recontamination of treated water between distribution and consumption has been linked to the spread of waterborne diseases among camp populations in multiple studies. At present, we have little evidence about which factors compromise or protect the safe water chain in refugee/IDP camps. We carried out a multi-site observational study to investigate how water handling behaviours,

water quality, and environmental factors affect post-distribution chlorine decay in order to inform best practices for preserving the safe water chain in refugee/IDP camps. In three refugee camps in South Sudan, Jordan, and Rwanda, we observed how water quality changed between distribution and consumption and documented water handling practices and environmental factors. We applied a multi-level modelling approach to elucidate relationships between chlorine decay and multiple predictor parameters. We found that environmental factors such as sunlight exposure strongly influenced the magnitude of post-distribution chlorine decay, while common water handling practices such as container covering did not yield a consistent protective effect. These findings can help humanitarian responders focus hygiene promotion efforts to best protect water safety in refugee/IDP camp settings.

1627

FEASIBILITY, ACCEPTABILITY AND SCALABILITY OF THE IMPLEMENTATION OF AN INTEGRATED WASH, NUTRITION, PREVENTION OF LEAD CONTAMINATION, AND CHILD STIMULATION THROUGH THE GOVERNMENT HEALTH SYSTEM IN BANGLADESH

Farzana Yeasmin[1], Mahbubur Rahman[1], Tania Jahir[1], Fahmida Akter[1], Mohammad Ruhul Amin[1], Jyoti Bhushan Das[1], Stephen P Luby[2], Peter J Winch[3]

[1]*International Centre for Diarrhoeal Diseases Research (icddr,b), Dhaka, Bangladesh,* [2]*Stanford University, California, CA, United States,* [3]*Johns Hopkins University, Baltimore, MD, United States*

We piloted an integrated intervention package of WASH, nutrition, prevention of lead contamination, and child stimulation to promote child growth and development in rural Bangladesh, and then revised the package. We implemented the revised package and explored the feasibility, acceptability and scalability of its implementation in one subdistrict (population approximately 291,121) through the government health system of Bangladesh. We conducted 17 in-depth-interviews with community health care providers, nine key informant interviews with supervisors and managers within the government health system, four focus group discussions with lactating and pregnant mothers and 12 in-depth interviews with lactating mothers. An early childhood development intervention package was effectively delivered through the government health services with high attendance. In our study many health care providers stated that though group sessions involved more time and effort compared to their existing activities but they could integrate it with their regular tasks. They were confident in conducting the sessions as they received training on how to deliver these sessions. The supervisors and managers mentioned that the majority of the service providers were motivated to conduct the sessions. They integrated the sessions with their regular working responsibilities. The mothers reported that the service providers showed a positive and friendly attitude to the participants. The mothers understood the session content and liked most of the intervention materials. They could actively participate in group sessions at various service delivery points. Most of the mothers stated that it was feasible for them to attend sessions. Most of the mothers stated that they could adopt the behavioral recommendations related to child stimulation. Respondents suggested that the integrated intervention could be implemented through the government health system of Bangladesh. As this was implemented within a government subdistrict healthcare system it would potentially be scalable, though it would require high-quality training and commitment from leadership.

1628

DECREASED BIOEFFICACY OF LONG-LASTING INSECTICIDAL NETS AND THE RESURGENCE OF MALARIA IN PAPUA NEW GUINEA

Rebecca J. Vinit[1], Lincoln Timinao[1], Nakei Bubun[1], Michelle Katusele[1], Leanne J. Robinson[2], Peter Kaman[1], Muker Sakur[1], Leo S. Makita[3], Lisa Reimer[4], Louis D. Schofiled[5], Ivo Mueller[6], William Pomat[1], Moses Laman[1], Tim Freeman[7], **Stephan Karl**[5]

[1]*Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea,* [2]*Burnet Institute, Melbourne, Australia,* [3]*Papua New Guinea National Department of Health, Port Moresby, Papua New Guinea,* [4]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [5]*Australian Institute of Tropical Health and Medicine, James Cook University, Cairns, Australia,* [6]*Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia,* [7]*Rotarians Against Malaria Papua New Guinea, Port Moresby, Papua New Guinea*

Papua New Guinea (PNG) has the highest malaria transmission outside of Africa and long-lasting insecticidal nets (LLINs) are the only vector-control tool distributed country-wide. LLINs were introduced into PNG around 2006 and have been attributed to have had very significant impact on the malaria burden, with reductions in observed average infection prevalence from 15.7% in 2008) to 1% in 2014. However, since 2015 malaria indicators in PNG have risen significantly. Similar trends have been observed in several African nations. In the present study, we observed a drastic reduction in bioefficacy of LLINs collected both from households as used nets and prior to use in original, unopened packaging. We hypothesise that decreased ability of LLINs to kill anopheline mosquitoes is a major contributor to the observed malaria resurgence in PNG and possibly in other parts of the world.New LLINs in original and unopened packaging (n=192) manufactured between 2007-2019 were collected in 15 PNG provinces. Used LLIN (n=40) manufactured between 2008 and 2017 were collected in 2 provinces. LLIN were subjected to standard World Health Organisation (WHO) cone bioassays using fully susceptible *An. farauti* mosquitoes. A subset of LLIN was re-tested using fully susceptible *An. gambiae* G3 mosquitoes in order to ensure reproducibility of results. Only 7% (95% CI 4-12%) of new LLINs manufactured between 2013-2019 exhibited 100% 24 h mortality when tested in cone bioassays. However, 84% (95% CI: 65-94%) new nets manufactured in 2012 or before exhibited 100% 24 h mortality. Only 29 % of used LLINs less than 3 years old exhibited > 80% 24 h mortality. Results obtained in tests using *An. farauti* corresponded well with confirmatory tests conducted using *An. gambiae*. Bioefficacy of LLINs in PNG appears to have been highly variable since 2013, with few LLINs manufactured since 2013 meeting WHO standards. This timeframe coincides with malaria resurgence in the country. These results may have ramifications for LLIN-based malaria control that go beyond the local PNG scenario.

1629

SLIPPING THROUGH THE NET: RELATIVE IMPACTS OF OWNERSHIP, RETENTION, AND USE ON INSECTICIDE-TREATED NET COVERAGE IN SUB-SAHARAN AFRICA

Amelia Bertozzi-Villa[1], Peter W. Gething[2], Caitlin Bever[1], Samir Bhatt[3]

[1]*Institute for Disease Modeling, Bellevue, WA, United States,* [2]*Telethon Kids Institute, Perth, Australia,* [3]*Imperial College, London, United Kingdom*

Insecticide-treated nets (ITNs) are one of the most powerful means of interrupting malaria transmission, yet virtually all malaria-endemic countries in sub-Saharan Africa did not achieve universal coverage targets in 2019. Effective ITN coverage relies on a complex political, economic, and cultural pipeline that includes sufficient distribution of nets by national programs, retention of nets in households for the duration of their effective lifetime, and consistent usage of nets throughout the year. We utilize a mixed-modeling strategy to show that ITN distribution and retention are larger barriers to effective coverage than ITN use, and to explore counterfactual mechanisms for increased coverage. We

first estimate true ITN distribution, retention, and use from 2000-2019, and subsequently explore the impact of three counterfactual scenarios: i) increasing the volume and frequency of ITN distributions to reach a larger portion of the population; ii) setting median ITN retention rates to three years everywhere from 2010 onward; and iii) setting ITN use given ownership to 100% everywhere. All models are run using an adaptation of the framework developed by Bhatt et al. (2015), which first uses a Gibbs-sampler-based mechanistic model to triangulate available data sources and produce estimates of country-specific ITN distribution and retention, and subsequently estimates ITN use via a series of geospatial regressions. We find that ITN distribution and retention have a complementary effect on overall coverage and increasing either could lead to substantial gains in ITN protection in SSA. While maximizing use rates also increases effective coverage, the relative gains in this scenario are not as large. However, our annual-level analysis may be masking important seasonal trends in ITN use. These results show the laudable impact of ITN community engagement campaigns on keeping use rates high, but also demonstrate the need for a deeper understanding of the motivations for ITN discarding, and new strategies for either increasing net distributions or encouraging net retention.

1630

EFFICACY OF TWO NEXT GENERATION LONG-LASTING MOSQUITO BED NETS (INTERCEPTOR® G2 AND ROYAL GUARD®) AGAINST PYRETHROID RESISTANT MALARIA VECTORS IN SOUTHERN BENIN; AN EXPERIMENTAL HUT EVALUATION

Corine A. Ngufor

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

Introduction: The effectiveness of pyrethroid mosquito bed-nets is threatened by widespread pyrethroid resistance in malaria vectors. A new generation of bed-nets containing a mixture of pyrethroids and novel compounds to which vector populations are susceptible, are being developed. Interceptor® G2 treated with chlorfenapyr and alphacypermerthrin and Royal Guard® treated with an insect growth regulator (pyriproxyfen) and alphacypermethrin are being evaluated in a community randomized clinical trial in Southern Benin. Method: We assessed the entomological efficacy of both bed-net types in human occupied experimental huts against pyrethroid resistant *An gambiae* sl in the clinical trial area. Comparison was made with Interceptor LN (an alphacypermethrin-only LLIN). The study was performed in 7 huts and lasted for 54 nights. Bed-nets were tested unwashed and after 20 standardized washes to mimic worn-out nets after 3 years of community use. Results: CDC bottle bioassays confirmed high levels of resistance to pyrethroids and full susceptibility to chlofenapyr and pyriproxyfen in the vector population. Mortality rates were low with Interceptor LN both before (34%) and after washing (30%). The highest levels of mosquito mortality were achieved with Interceptor G2 (74%) and this did not decline after 20 washes (72%, P>0.05). Royal Guard provided improved levels of mortality compared to Interceptor LN when unwashed (46% vs. 34, P<0.05) but not after 20 washes (32% vs. 30% P>0.05). Both next generation nets induced improved levels of blood-feeding inhibition compared to Interceptor LN both before and after washing. Royal Guard also sterilized all blood-fed pyrethroid resistant mosquitoes which survived exposure to the net in the hut trial when unwashed (100% oviposition inhibition) and after 20 washes (42% oviposition inhibition). Conclusion: Interceptor G2 and Royal Guard show potential to provide improved protection against clinical malaria compared to pyrethroid-only nets in Southern, Benin.

1631

ASSESSING THE NON-INFERIORITY OF NOVEL INSECTICIDE-TREATED NETS IN EXPERIMENTAL HUT TRIALS

Joseph D. Challenger[1], Rebecca K. Nash[1], Mark Rowland[2], Corine Ngufor[2], Raphael N'Guessan[3], Antoine Sanou[4], Fatoumata Cissé[4], Richard M. Oxborough[5], Sarah J. Moore[6], Thomas S. Churcher[1]

[1]*Imperial College London, London, United Kingdom,* [2]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [3]*Institut Pierre Richet, Bouaké, Côte D'Ivoire,* [4]*Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso,* [5]*PMI-VectorLink Project, Rockville, MD, United States,* [6]*Ifakara Health Institute, Bagamoyo, United Republic of Tanzania*

Insecticide-treated nets (ITNs) have been the cornerstone of malaria control for decades, especially in Sub-Saharan Africa. ITN efficacy is diminishing across the continent due to high levels of pyrethroid resistance in mosquito populations meaning that new ITN products are urgently needed. Experimental hut trials can be used to demonstrate the efficacy of ITNs on mosquito populations in endemic settings and can evaluate whether novel products are non-inferior to existing ITNs which have a proven public health value. In this work, we examine how such trials should be carried out to ensure that assessments of non-inferiority are adequately powered. We explore the implications of several sources of variation present in experiment hut trials, such as variation introduced by rotating nets over different huts in the trial. A statistical framework is devised to robustly evaluate novel products and the accuracy of the assay is investigated using data from a meta-analysis of ITNs. It is unclear what the minimum acceptable inferiority of a new product should be. Here we investigate this quantity taking into account the accuracy of the experimental hut trial and the public health impact that an inferior product could have using a model of malaria transmission.

1632

BEHAVIORAL BIOASSAYS TO IMPROVE BEDNET EVALUATION IN THE LABORATORY AND FIELD

Geraldine M. Foster[1], Agnes Matope[1], Amy Guy[1], Mischa Emery[1], Keith Steen[1], Katherine Gleave[1], Jeff Jones[1], David T. Towers[2], Hilary Ranson[1], Philip J. McCall[1]

[1]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [2]*University of Warwick, Warwick, United Kingdom*

Insecticide treated nets (ITNs) are essential for sustainable reduction and eventual elimination of malaria in sub-Saharan Africa but are losing efficacy as pyrethroid resistance increases. With the introduction of dual active ingredient ITNs into pilot deployments and randomised control trials, methods to characterise the efficacy of differing modes of action on disparate vector populations are urgently required. We have developed a suite of bioassays capable of capturing diverse modes of action that are suitable for screening ITNs during development from early stage screening for insecticidal effects to evaluating performance of the ITN *in situ* at affected communities in Africa. This behavioural test suite avoids forced contact, instead simulating exposure under more natural conditions, usually with the inclusion of a human host attractant. There are three systems, Video Cone Test Assay (ViCTA), Baited Box test and Room-scale Video Tracking, each permitting characterisation at different levels of detail from rapid screening for repellent or irritant effects, to characterisation of late stage host seeking at the human-ITN interface, to visualisation of vector activity around the entire host-net environment. All generate a video record and data variously on knockdown, mortality at 24, 48+ hrs, repellency, contact irritancy, duration and distribution of net contact, blood feeding (duration, volume, rate, inhibition) and temporal changes in these responses. Maintaining mosquitoes post-exposure allows detection of delayed or sub-lethal effects *e.g.* refeeding ability, oviposition and hatching rates, and longevity until natural death and allows harmonisation of data between laboratory and semi-field experiments. Together with data from experimental hut trials, test outputs greatly improve evidence-

based predictions regarding the likely success of new ITN products against specific vector populations, increasingly important for decision-makers as the choice of net types grows. Video and new data will be used to demonstrate the ease and benefits of incorporating behavioural bioassays into bednet evaluation.

1633

COMMUNITY PERCEPTION OF THE USE OF NEXT-GENERATION INSECTICIDE-TREATED BEDNETS IN RURAL AREAS IN THE HEALTH DISTRICTS OF BANFORA, ORODARA AND GAOUA IN BURKINA FASO: BASELINE DATA

Moubassira Kagone[1], Abdoulaye Traore[1], Federica Guglielmo[2], Adama Traore[1], Samuel Poda[1], Aboubacar Fofana[1], Kenzie Tynuv[3], Moussa Guelbeogo[1], N'Fale Sagnon[1], Christelle Gogue[3], Adama Gansané[1], Molly Robertson[3]

[1]*Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso,* [2]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [3]*PATH, Washington, DC, United States*

In response to the emergence and intensification of insecticide resistance in key mosquito populations, next-generation insecticide-treated bednets (ITNs) that are effective against insecticide resistant mosquitoes have been developed. As part of the New Nets Project (funded by a partnership between Unitaid and The Global Fund), observational studies are accompanying the piloted Interceptor G2® (IG2)(BASF) rollout in order to collect data on their entomological and epidemiological impact and on anthropological factors that influence their uptake and usage. Anthropological data collection was conducted in three health districts: Banfora, Orodara, and Gaoua, Burkina Faso, from July to September 2019. We used a combination of in-depth interviews (n=146), structured observations (n=217), participant observations (n=469), and focus groups discussions (n=36). The study participants were mothers of children under the age of five, pregnant women, heads of households from different socio-professional occupations, and community leaders. A thematic analysis was conducted. All interviews were transcribed and analysed using NVivo software (QSR International Inc., Burlington, MA, USA). Preliminary results show that most of the respondents were familiar with malaria, commonly known as "soumayaba" in the local Dioula language, a disease that is believed to be transmitted by mosquito bites. The symptoms of the disease are also known by many people. Recurrent signs cited are fever, vomiting, cold, loss of appetite, and headaches. Therapeutic recourse for treatment remains both modern and traditional care. One of the means cited by all the respondents to protect themselves from mosquito bites remains the ITN. In addition to the use of ITNs, some participants also use insect spray. Taking community perceptions into account when distributing ITNs will be critical to improving effective usage and reducing malaria morbidity and mortality.

1634

A PILOT STUDY TO EVALUATE THE EFFECT OF NEXT-GENERATION INSECTICIDE-TREATED BEDNETS ON MALARIA MORBIDITY IN THREE HEALTH DISTRICTS IN BURKINA FASO: PRELIMINARY RESULTS OF THE BASELINE CROSS-SECTIONAL SURVEY

David Kangoye[1], Christelle Gogue[2], Siaka Debe[1], René Kinda[1], Adama Ganou[1], Casimir Tarama[1], Harouna Sore[1], Cheick S. Compaore[3], N'Fale Sagnon[1], Moussa Guelbeogo[1], Molly Robertson[2], Adama Gansané[1]

[1]*Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso,* [2]*PATH, Washington, DC, United States,* [3]*Programme National de Lutte contre le Paludisme, Ouagadougou, Burkina Faso*

World Health Organization (WHO) targets for 2030 include a 90% reduction in malaria morbidity and mortality compared to the 2015 baseline. However, achievement of these goals is threatened by the emergence of resistance in both the parasite to antimalarial drugs and in the vector to insecticides. To overcome vector resistance, several new insecticide-treated bednets (ITNs) with new insecticide formulations and evidence of laboratory and experimental hut efficacy have been prequalified by the WHO. As part of the New Nets Project (funded by a partnership between Unitaid and The Global Fund), observational studies are accompanying the piloted Interceptor® G2 ITN (BASF) (IG2) rollout in order to collect data on their entomological and epidemiological impact and on anthropological factors that influence their uptake and usage. In Burkina Faso, serial cross-sectional studies of malaria prevalence before and after the rollout of a next-generation ITN will provide information on impact. The preliminary results of the baseline cross-sectional survey conducted in July 2019 are presented here. A sample of 190 households per district in the three selected health districts (Banfora, Gaoua, and Orodara) was included in the survey. The parasite prevalence in children 6 months to 5 years old was determined by rapid diagnostic tests, and questions were asked about the possession and use of ITNs. Of the three pilot districts, Gaoua had the highest parasite prevalence (81% , χ^2 = 126.8, p = 0.0001), the lowest net possession rate (69%, χ^2 = 307.7, p = 0.0001) and the lowest net-use rate (17.8% χ^2 = 201.02, p = 0.0001). The study team will also conduct further analysis to identify risk factors for infection and low net-use along with potential solutions.

1635

MULTI-CENTRIC FIELD EVALUATION OF A DIGITAL MALARIA MICROSCOPY DEVICE BASED ON MACHINE-LEARNING: EASYSCAN GO - A PRELIMINARY ANALYSIS

Debashish Das[1], Ranitha Vongpromek[2], Thanawat Assawariyathipat[2], Ketsanee Srinamon[3], Kalynn Kennon[1], Kasia Stepniewska[1], Aniruddha Ghose[4], Abdullah Abu Sayed[4], Rebeca Netto[5], Andre Siqueira[5], Serge R Yerbanga[6], Jean Bosco Ouédraogo[6], James Callery[3], Rupam Tripura[3], Felix Koukouikila Koussounda[7], Francine Ntoumi[7], John Michael Ongecha[8], Bernhards Ogutu[8], Jutta Marfurt[9], Benedikt Ley[9], Amadou Seck[10], Magatte Ndiaye[10], Laypaw Archasuksan[11], Stephane Proux[11], Sam L Nsobya[12], Philip J. Rosenthal[13], Matthew Horning[14], Shawn K McGuire[14], Courosh Mehanian[14], Stephen Burkot[14], Christine Bachman[14], Richard N. Price[9], Arjen Dondorp[15], Philippe J Guérin[15], Mehul Dhorda[15]

[1]*Infectious Diseases Data Observatory (IDDO) & WorldWide Antimalarial Resistance Network (WWARN), Oxford, United Kingdom,* [2]*Infectious Diseases Data Observatory (IDDO) & WorldWide Antimalarial Resistance Network (WWARN), Asia-Pacific Regional Centre, Bangkok, Thailand,* [3]*Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand,* [4]*Chittagong Medical College Hospital (CMCH), Chittagong, Bangladesh,* [5]*Oswaldo Cruz Foundation (Fiocruz), Rio de Janeiro, Brazil,* [6]*Institut des Sciences et Techniques (INSTech), Bobo-Dioulasso, Burkina Faso,* [7]*Fondation Congolaise pour la Recherche Médicale (FCRM), Brazzaville, Republic of the Congo,* [8]*Kenya Medical Research Institute (KEMRI), Nairobi, Kenya,* [9]*Global and Tropical Health Division, Menzies School of Health Research, Charles Darwin University, Darwin, Australia,* [10]*Faculty of Medicine, University Cheikh Anta Diop (UCAD), Dakar, Senegal,* [11]*Shoklo Malaria Research Unit, Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Mae Sot, Thailand,* [12]*Department of Pathology, College of Health Science, Makerere University, Kampala, Uganda,* [13]*University of California, San Francisco, San Francisco, CA, United States,* [14]*Intellectual Ventures' Global Good Fund, Bellevue, WA, United States,* [15]*Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom*

Microscopic examination of Giemsa-stained blood films remains the reference standard for laboratory confirmation of malaria but is undermined by difficulties in ensuring high quality manual reading and inter-reader reliability. Automated parasite detection and quantification may address this issue. We assessed the performance of the EasyScan Go, a microscopy device employing machine learning-based image analysis to detect malaria parasites. A prospective study was conducted during 2018 and 2019 at 10 sites in 10 countries from Africa, Asia and

South America. Giemsa-stained blood films were prepared and read by expert microscopists and the EasyScan Go device. A selection of slides were rechecked for quality control. Of 2110 patients enrolled, 919 tested positive by expert microscopy of which 62% (n=574) were infected with *P.falciparum*, 36% (n=327) with *P.vivax* and 2% (n=18) with mixed infections. Diagnostic sensitivity (Se) of the EasyScan Go device was 91.1% (95%CI:88.9-93.0%) and specificity (Sp) was 73.6% (95%CI:70.8-76.3%). Se varied according to parasite density - 59% at <200 p/µL, rising to ≥90% at densities >200-200000 p/µL. Parasite species were identified accurately in 91% *P. falciparum* samples (362/399; kappa = 0.73) and in 92% *P. vivax* samples (281/307; kappa = 0.71). Intraclass correlation coefficient for parasite density estimates obtained from comparison of manual microscopy with the EasyScan GO was 0.33 (95%CI:0.26-0.39) indicating moderate agreement. When results from sites assessed to have low quality slides during quality control with respect to smear and/or stain quality were excluded, Se decreased to 88.6% (95%CI:85.2-91.5%) but Sp improved to 84.7% (95%CI:81.8-87.3%). Further software improvement is required to improve parasite density estimations and Se at low parasite densities. High quality of smears and staining is paramount to allow machine learning-based image analysis to perform adequately.

1636

A NEW BIOINFORMATIC PIPELINE FOR IDENTIFYING DIAGNOSTIC-RESISTANT *PLASMODIUM FALCIPARUM* WITH *HRP2/3* DELETIONS USING LONG-READ SEQUENCING TECHNOLOGY

Camille E. Morgan[1], Jonathan B. Parr[2], Kara A. Moser[2], Chris M. Hennelly[2], Jonathan J. Juliano[2], Corbin D. Jones[3], Jeremy R. Wang[4]

[1]*Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States,* [2]*Institute for Global Health and Infectious Diseases, University of North Carolina Chapel Hill, Chapel Hill, NC, United States,* [3]*Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States,* [4]*Department of Genetics, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States*

Rapid diagnostic tests (RDT) are a critical component of treating the estimated 219 million cases of malaria worldwide, most of which occur in settings with few healthcare resources. The most common RDT used for diagnosis of *P. falciparum* (*Pf*) malaria parasites detects histidine-rich proteins 2 and 3 (HRP2/3), non-essential proteins secreted in high levels. Reports of *hrp2/3*-deleted strains are increasing globally, and improved methods are needed to detect and study such strains. Short-read next-generation sequencing is inefficient at detecting structural variations. Long-read sequencing using the Oxford Nanopore Technology (ONT) MinION platform offers a more robust method for studying structural variants and subtelomeric genes, like *hrp2/3*. We developed a computational pipeline to assess *hrp2/3* deletion status using ONT reads. We generated sequence data from the DD2 (*hrp2*-negative) and 3D7 (*hrp2*-positive) lab strains with >500x coverage, and we created positive and negative matches for *hrp2* deletions. We repeated this for *hrp3* using long reads of the *hrp3*-negative HB3 lab strain available from NCBI. We tested accuracy by subsampling reads to assess lower coverage levels and applied the pipeline to seven *Pf* isolates sequenced with long-read technology (ONT or PacBio) and publicly available on NCBI, all of which have intact *hrp2/3* genes with the exception of HB3. Our method correctly identified *hrp2/3*-status at as low as 5x coverage and accurately called *hrp2/3*-status for all seven NCBI long-read sequences. Efforts to apply this pipeline to long-read sequencing data generated using novel enrichment methods for *Pf* genomic material in field samples is underway, including assessment of its ability to call *hrp2/3* deletions in mixed infections and in samples with diverse deletion breakpoints. Improved long-read sequencing methods and analysis pipelines will improve our understanding of the biology and evolution of *hrp2/3*-deleted *Pf* strains.

1637

FIGHTING MALARIA, ONE IMAGE AT A TIME: THE DESIGN AND PRELIMINARY VALIDATION OF A LOW-COST FIELD TOOL FOR THE RAPID AND ACCURATE MORPHOLOGICAL IDENTIFICATION OF MALARIA VECTORS

Sophia Diaz[1], Monet Slinowsky[1], Kiley Gersch[1], Ebenezer Armah[1], Karina Frank[1], Zachary Buono[1], Tristan Ford[1], Adam Goodwin[2], Margaret Glancey[1], Soumyadipta Acharya[1]

[1]*Johns Hopkins University, Baltimore, MD, United States,* [2]*VecTech, LLC, Baltimore, MD, United States*

Vector control is the mainstay of malaria prevention. It depends on the rapid and accurate identification of malaria vectors from collected specimens, which directs the distribution of species-specific interventions. Unfortunately, the taxonomic expertise needed to achieve this is often unavailable, forcing programs to use inadequate data for decision-making or resort to costly molecular identification methods. Our team developed a tool capable of capturing high-resolution images of mosquito specimens and identifying the primary malaria vectors found in sub-Saharan Africa using a computer vision algorithm. The algorithm is based on Deep Learning Convolutional Neural Networks (CNNs) trained on a database of over 12,000 field-caught mosquitoes. Mosquito species were grouped into six categories: Aedes, Culex, Anopheles Gambiae, Anopheles Funestus, "Anopheles other," and "other." Prior work in our lab determined 16 line pairs per millimeter (lp/mm) as the minimum image resolution required for entomologists to assess key morphological features differentiating mosquito species. The team tested various optical configurations using off-the-shelf components. The lighting, lenses, and resultant resolutions were optimized to determine the design specifications necessary to build a low-cost, high-resolution imaging system. In addition, the algorithm was tested using a novel set of mosquito images. The tool achieved 20-34 lp/mm resolutions, surpassing the minimum required resolution. The algorithm identified primary malaria vectors to an overall accuracy of 95.6%. It identified the individual categories with the following accuracies: 83.1% for Anopheles Gambiae, 100.0% for Anopheles Funestus, 99.0% for "Anopheles other," 97.0% for Culex, 96.3% for Aedes, and 94.6% for "other". Full set results are pending. Implemented in a simple imaging tool coupled with novel computer vision algorithms, this technology significantly outperforms manual identification methods, improving the current practice by simplifying vector surveillance and accurately informing downstream control interventions that prevent disease.

1638

STRUCTURE-SWITCHING APTAMER SENSORS FOR THE SPECIFIC DETECTION OF PIPERAQUINE AND MEFLOQUINE

Erin S. Coonahan[1], Kyung-Ae Yang[2], Maarten De Vos[3], Stevan Pecic[4], Joel Tarning[5], Thomas E. Wellems[1], John F. Andersen[1], Carole A. Long[1]

[1]*Laboratory of Malaria and Vector Research, NIAID, NIH, Rockville, MD, United States,* [2]*Department of Medicine, Columbia University, New York, NY, United States,* [3]*Institute of Biomedical Engineering, University of Oxford, Oxford, United Kingdom,* [4]*Department of Chemistry and Biochemistry, California State University Fullerton, Fullerton, CA, United States,* [5]*Clinical Pharmacology, Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand*

Tracking antimalarial drug use and efficacy in Southeast Asia is important for monitoring the spread of drug resistant parasites. However, current methods for assessing patient drug levels and tablet quality are often inaccessible, as they require well-equipped laboratories capable of performing LC-MS. This research aimed to develop a rapid, aptamer-based fluorescent sensor for the specific detection of the antimalarial compounds piperaquine and mefloquine. These compounds are slow-clearing partner drugs in current first-line artemisinin combination therapies (ACTs). DNA aptamers were identified that bind piperaquine (K_D = 0.9 nM) and mefloquine (K_D = 19 nM) with high selectivity over similarly structured small molecules. The aptamers were selected from a library of single-

stranded DNA then adapted into structure-switching aptamer fluorescent sensors. Sensor performance was optimized for the detection of drug from crushed tablets and from human plasma. The sensors were evaluated for their sensitivity and specificity in relevant sample matrices and the plasma platform was validated against an LC-MS standard drug detection method as well as in patient samples. This assay provides a rapid and inexpensive method for tracking antimalarial drug quality and use at a time when the containment and study of parasite resistance is a major priority for malaria elimination campaigns. This sensor allows for flexibility of sample matrix and can be easily adapted for the detection of other small molecule drugs.

1640

DEVELOPMENT OF THE 1ST WORLD HEALTH ORGANIZATION INTERNATIONAL STANDARD FOR *PLASMODIUM VIVAX* ANTIGENS

Charles Olomu[1], Lynne M. Harris[1], **Seda Yerlikaya**[2], Peter Rigsby[1], Eleanor Atkinson[1], Adéla Nacer[1], Xavier Ding[2], Paul W. Bowyer[1], the Collaborative Study Group[2]

[1]*National Institute for Biological Standards and Control (NIBSC), Blanche Lane, South Mimms, Hertfordshire, United Kingdom,* [2]*FIND, Geneva, Switzerland*

A suite of WHO reference materials for *Plasmodium falciparum* such as the first International Standard (IS) for *P. falciparum* antigens and a Reference Reagent for serology have been established to standardize *P. falciparum* assays. *P. vivax*, the second most common human-infecting *Plasmodium* species, is lacking International Standards. FIND and NIBSC have recently completed an international collaborative study toward the establishment of the first WHO IS for *P. vivax* antigens. The study results will be presented to the 71st meeting of the WHO Expert Committee on Biological Standardization (ECBS). A candidate material consisting of lyophilized red blood cell lysates from *P. vivax*-infected donors was prepared. An international collaborative study involving 16 participant laboratories in 11 countries evaluated the suitability of the candidate IS in a range of *P. vivax* antigen detection assays alongside *P. vivax* clinical isolates and recombinant *P. vivax* lactate dehydrogenase (PvLDH). An accelerated thermal degradation study was conducted in parallel to assess the suitability of the standard in terms of its predicted long-term stability. The collaborative study found that the candidate IS was detectable on all 18 rapid diagnostic tests (RDTs) studied. The candidate showed the same qualitative behavior as the geographically diverse clinical isolates. Reporting limit of detection titer relative to the candidate effectively harmonized RDT data between labs. The candidate standard performed less well in ELISAs, but so did the clinical isolates tested. Nonetheless, it was determined that reporting potency relative to the IS gave a large reduction in the geometric coefficient of variation (GCV) from a range 81.3 - 274.7% to 13.9 - 31.3%. Early accelerated thermal degradation results indicated good long-term stability. We will present the results of the characterization of the candidate IS and the collaborative study, together with the outcome of the ECBS meeting. Development of an IS for *P. vivax* antigen will support quality control and standardization of malaria RDTs worldwide and facilitate the development of more sensitive diagnostic tests.

1641

DEVELOPING A SEROLOGICAL RAPID TEST FOR AN UNMET DIAGNOSTIC NICHE: THE *PLASMODIUM VIVAX* HYPNOZOITE

Rosalind E. Howes[1], Anya Gregg[2], Matthias Harbers[3], Andrew Lover[4], Agatha Mia Puspitasari[5], Rintis Noviyanti[5], Jutta Marfurt[6], Ric Price[6], Sabine Dittrich[1], Marta Fernandez Suarez[1], Xavier C. Ding[1]

[1]*Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland,* [2]*Mologic, Bedford Technology Park, United Kingdom,* [3]*CellFree Sciences Co. Ltd., Yokohama, Japan,* [4]*Dept of Biostatistics and Epidemiology, School of Public Health and Health Sciences, University of Massachusetts-Amherst, Amherst, MA, United States,* [5]*Eijkman Institute for Molecular Biology, Jakarta, Indonesia,* [6]*Menzies School of Health Research, Darwin, Australia*

Plasmodium vivax forms persistent parasites in the liver which undermine conventional malaria elimination interventions by causing repeated relapses in the months following the mosquito-borne infection. These relapses cause a cumulative burden of harm on the infected patient and represent opportunities for onward transmission. The liver parasites, known as hypnozoites, are the predominant source of blood-stage infections and thus represent an important diagnostic target for identifying parasite reservoirs. Direct detection of hypnozoites in the liver is currently not feasible, but serological approaches could provide evidence of recent exposure. Discovery studies identified *P. vivax* antigens associated with specific antibody responses that are indicative of recent past infection and, by extension, of a high likelihood of hypnozoite presence. The best performing of these antigens was reticulocyte binding protein 2b (PvRBP2b). Here we describe ongoing efforts to transfer this promising antigen into a lateral flow rapid test for exposure to *P. vivax* in the last nine months. Given the remote rural settings where *P. vivax* persists, the design needed to provide results from capillary blood within 10 minutes, without specialized equipment or training. A recombinant protein construct of a PvRBP2b fragment was expressed in a wheat germ cell-free system and integrated into a sandwich assay device to detect the specific IgG antibody biomarker of recent infection. Performance evaluation studies are ongoing to assess the prototype's analytical sensitivity and specificity (capacity to detect the target antibodies against a reference assay result). In parallel, the test's capacity to detect past *P. vivax* infection is being assessed with 1352 banked plasma samples from a cohort of patients with documented malaria clinical histories from Papua, Indonesia. The example of this candidate test and considerations for its future development will be discussed in the context of an overview of the regulatory processes for in-vitro diagnostics.

1642

REASONS FOR INSUFFICIENT PREVENTIVE CHEMOTHERAPY COVERAGE FOR NEGLECTED TROPICAL DISEASES, A MULTI-COUNTRY ANALYSIS

Sabrina Eyob[1], Alexander H. Jones[1], Margaret Baker[1], Alfred Mubangizi[2]

[1]*RTI International, Washington, DC, United States,* [2]*Vector Control Division, Ministry of Health, Kampala, Uganda*

The World Health Organization has established disease-specific preventive chemotherapy (PC) coverage targets for trachoma, lymphatic filariasis (LF), onchocerciasis, soil-transmitted helminthiasis (STH), and schistosomiasis. From 2017-2019, USAID and partners supported nine national neglected tropical disease (NTD) programs to implement 3,503 district-level mass drug administrations (MDAs) in 830 districts. To understand and address the reasons for MDAs not meeting PC coverage targets, we investigated each MDA that had insufficient coverage and documented the main causes. From 2017-2019, 314 (9%) MDAs in seven (78%) countries and 193 (23%) districts did not meet coverage targets. The number of low coverage MDAs decreased from 154 (12%; n=1,242) in 2017 to 81 in 2019 (8%; n=1,038). Each PC NTD had ≥1 low coverage MDA; 134 were for STH (43%) followed by 69 (22%) for schistosomiasis. In 2017, 47 (31%) LF MDAs had low coverage, but by 2019 the disease accounted for only 4 (5%) such MDAs. Notably, among all diseases, 43 (22%) districts had low coverage in ≥2 consecutive years. Late or insufficient drug supply was the most common (24%, n=314) reason for low coverage overall. In 2019 alone, drug supply problems led to 58 (72%) low coverage MDAs, all in one country. Also, reporting issues and community resistance to ingesting the drug were major reasons for low coverage each year. They accounted for 59 (19%) and 54 (17%) low coverage MDAs, 2017-2019. In 2017, reporting issues caused 38 (25%) low coverage MDAs, but by 2019 reporting was the primary cause of only 5 (6%) such MDAs. Similarly, the number of MDAs with community resistance as the main cause of low coverage decreased from 39 (25%) to 7 (9%). National

NTD programs and partners should continue targeted support to districts that consistently had low coverage MDAs. It seems obvious, but is worth highlighting, that national-level issues (e.g. drug shortages) will impact coverage in more districts than local-level issues. Local issues will continue to require attention as NTD programs mature and as programs approach their goals of elimination or sustainability.

1643

USING PARTICIPATORY METHODS TO IMPROVE NTDS PROGRAMME OUTCOMES IN NIGERIA AND TO INFORM INTERVENTION DESIGN

Martins Imhansoloeva[1], Laura Dean[2], Ruth Dixon[3], Margo Greenwood[3], Shahreen Chowdhury[2], Catherine Kajang[1], Celestine Njoku[4], Sunday Isiyaku[1]

[1]*Sightsavers Nigeria, Abuja, Nigeria,* [2]*Liverpool School of Tropical Medicine, Liverpool, United Kingdom,* [3]*Sightsavers UK, Haywards Heath, United Kingdom,* [4]*Federal Ministry of Health, Abuja, Nigeria*

Integrated case management of NTDs has been adopted as a strategy to improve early case detection and management to reduce the disease burden in Nigeria. Often however, the opinions and perceptions of beneficiaries and implementers of these programs at the grassroots get lost and not reflected in its design. This research captured the voices of frontline health workers and people in rural areas affected by NTDs; it considers how their experiences of accessing these services can be used to shape program design and implementation. A community-based participatory research (CBPR) approach was used. People affected by NTDs and health workers were recruited as co-researchers to collect data. Analysis was completed through a series of participatory workshops where affected persons and health workers collaboratively 1) identified the emerging themes from the transcripts using the Levesque framework for care seeking; 2) fed back findings to program planners using cartoons designed by a local artist, and 3) prioritized areas for intervention to feedback into NTD programs. We collected 53 illness narratives among people affected by NTDs and their careers, and conducted 6 focus group discussions among people affected by NTDs and 24 with community health workers. Participants identified two main intervention areas to improve access and delivery of integrated services for persons affected with NTDs to include updated sensitisation messaging that address community perceptions and fosters community support rather than targeting disease "knowledge"; and secondly updated health worker training focussing on community based care options and soft skills such as gender sensitivity during service delivery. Both recommendations are currently being implemented with co-planning between implementers and co-researchers. The CBPR approach stimulated new thinking around the delivery of NTD programmes and responsiveness to findings, as well as provided the platform to engage with program planners and policy makers to advocate for tailored healthcare service with increased ownership.

1644

BROKERED DESIGN: A NOVEL METHOD FOR DESIGNING (AND RE-DESIGNING) NTD ELIMINATION PROGRAMS

Lee T. Wilkers[1], Luccene Desir[2], Gregory S. Noland[2], James V. Lavery[1]

[1]*Rollins School of Public Health, Emory University, Atlanta, GA, United States,* [2]*The Carter Center, Atlanta, GA, United States*

To be effective, disease elimination activities, such as mass drug administration (MDA) campaigns for neglected tropical diseases (NTD), require high adherence rates and the effective navigation of a complex array of stakeholder (SH) interests. Current implementation manuals offer limited guidance about how insights from local SHs can be collected and integrated into the design and day-to-day management of elimination programs. Human-Centered Design (HCD) and 'design thinking' strategies have been employed by many global health programs, but they often fail to elicit the kind of insights about SH interests that might provide the most valuable guidance. Elsewhere, we have described SH interests as "design parameters" that can serve as navigation aids by global health programs. By failing to fully account for the relevant SH interests, global health programs can underestimate the social complexity of the contexts they work within, miss opportunities to create value, and fail to recognize obligations to avoid setting specific SH interests back, i.e., harming them. *Brokered Design* is a novel method for efficiently capturing insights from discrete groups of SHs and feeding those insights into program design and management processes. The central feature of the *Brokered Design* method is the "2-way dashboard", a program management tool for presenting program and SH interests to facilitate dialogue, deliberation, and negotiation around key issues to maximize user desirability, technical feasibility, and economic viability. Here, we describe the *Brokered Design* method and our experience using it to refine the Lymphatic Filariasis MDA program in Léogâne, Haiti to improve participation rates. We outline the method's procedures and consider its context-dependent strengths and limitations. While our findings derive from a single NTD elimination context, we describe *Brokered Design*'s applicability to broader disease elimination efforts and complex global health challenges where effective SH engagement is critical for success.

1645

INFORMATION SHARING AND SUPPLY CHAIN PERFORMANCE: EVIDENCE FROM THE NEGLECTED TROPICAL DISEASES PREVENTIVE CHEMOTHERAPY SUPPLY CHAIN

Elena Kasparis[1], Yufei Huang[2], William Lin[1], Christos Vasilakis[1]

[1]*University of Bath, Bath, United Kingdom,* [2]*Trinity College Dublin, Dublin, Ireland*

Public-private partnerships contribute billions of donated medicines to mass drug administrations in support of the World Health Organization's "Roadmap to Implementation," which outlines targets to control, eliminate, and eradicate Neglected Tropical Diseases (NTDs). NTDs are infectious diseases afflicting over one billion people, principally the world's poorest—causing disfigurement, disability, and blindness. The supply chain to deliver donated preventive chemotherapy is complex due to many partners involved and further complicated by the need to deliver to remote destinations in developing countries. Fragmented data systems and limited transparency on supply chain performance caused additional challenges. Delivery was performing below standards, lagging quite below the WHO target for 80% on-time delivery, leading to untreated patients and waste. In September 2016, an online supply chain performance measurement system (SCPMS), "NTDeliver," was launched by the NTD Supply Chain Forum to enhance supply chain performance information transparency. The aim of the research is to empirically assess whether and how the SCPMS improved performance. Secondary data was collected from the SCPMS covering 1,400+ shipments for four critical medications delivered to over 100 countries. We applied regression models to assess impact on performance, comparing historical data before the SCPMS to post SCPMS launch. The results suggest information sharing has had a positive impact on three performance indicators: purchase order timeliness, arrival timeliness, and—most importantly—delivery timeliness. Our analysis suggests more substantial, positive impact when information is publicly accessible, geared towards country program managers. These results may support the WHO, pharmaceutical companies, and implementing countries to manage the supply chain more effectively and increase understanding of leveraging information to drive performance. More broadly, the research supports investment in information sharing in humanitarian supply chains and data transparency with staff managing shipment logistics in-country.

1646

EFFORTS TOWARDS STRENGTHENING THE INTEGRATION OF SUPPLY CHAIN OF NEGLECTED TROPICAL DISEASE MEDICINES INTO THE ELECTRONIC LOGISTICS MANAGEMENT INFORMATION SYSTEM IN TANZANIA

Frank Komakoma[1], Upendo Mwingira[2], Jeremiah Ngondi[2], Daudi Msasi[3], William Reuben[3], Nabila Hemed[3], Godfrey Kingalu[3], Kerry Dobbies[4], Elisabeth Wilskie[5], Abdallah Ngenya[6], Oscar Kaitaba[6], George Kabona[6]

[1]*IMA World Health, Dar es Salaam, United Republic of Tanzania,* [2]*RTI International, Washington, DC, United States,* [3]*Pharmaceutical Services Unit-MoHCDGEC, Dodoma, United Republic of Tanzania,* [4]*IMA World Health, Washington, DC, United States,* [5]*PATH, Dar es Salaam, United Republic of Tanzania,* [6]*MoHCDGEC, Dar es Salaam, United Republic of Tanzania*

Tanzania's supply chain management (SCM) of neglected tropical disease (NTD) medicines has gone through stages of improvement to achieve goals of effective mass drug administration (MDA). Initial assessment of the supply chain in 2014 revealed these challenges: poor inventory leading to shortages during MDA; large quantities of medicines remaining after MDA; medicines expiring before next MDA; and poor storage conditions compromising quality of medicines. In 2014, the NTD program together with partners developed supply chain guidelines for community drug distributors, frontline health workers and district pharmacists. Guidelines aimed to support NTD teams to better manage inventory, quantify medicines accurately, improve adverse effects reporting, and conduct reverse logistics. Following implementation of the guidelines, the program saw improvements in management of medicines whereby districts reported having fewer drugs in stock after MDA. After the 2014 MDA for example, a total of 3,419 bottles of Zithromax tabs and 84,256 bottles of Zithromax oral solution remained at district level. In 2018, this was down to 504 bottles of Zithromax tabs and 28,533 bottles of Zithromax oral, representing a combined 66% reduction in four years. The second improvement started in 2018 and aimed at streamlining inventory management among various stakeholders including the NTD program, Medical Stores Department (MSD), Pharmaceutical Services Unit, district and health facilities. This involved inclusion of NTD medicines at all levels into the electronic Logistics Management Information System (eLMIS) which collects and provides logistics data in real time. As a result, the streamlined eLMIS will enable health facilities to order NTD drugs directly at MSD and strengthen ownership at the community level; MSD to plan efficient distribution of NTD medicines to health facilities; and NTD program to view quantities of medicines available at all levels after MDA and correctly quantify amounts required. It also allows for more efficient planning of reverse logistics, more accurate data, and better monitoring of expired medicines.

1647

LOCAL TIPS, GLOBAL IMPACT: COMMUNITY-DRIVEN SOLUTIONS FOR NEGLECTED TROPICAL DISEASES (NTDS) IN SUB-SAHARAN AFRICA: A CASE STUDY OF KENYA

Elizabeth Akinyi Ochola[1], Diana M. Karanja[2], Susan J. Elliott[3]

[1]*Kenya Medical Research Institute/ University of Waterloo, Kisumu, Kenya,* [2]*Kenya Medical Research Institute, Kisumu, Kenya,* [3]*University of Waterloo, Waterloo, ON, Canada*

Neglected Tropical Diseases (NTDs) remain common to many regions of sub-Saharan Africa (SSA) left behind by socioeconomic progress. As such, these diseases affect populations that have little or no 'political voice' to influence NTD control activities. As countries embrace and work towards achieving the Sustainable Development Goals (SDGs), the needs of such marginalized populations need to be addressed in the local and global arenas. Even though most NTDs have available interventions that work, the biggest challenge remains on how to successfully engage communities and advance context specific solutions to NTDs, especially in areas that experience weak health systems. As such, this research investigated the capacity of local communities to address the burden of NTDs. Informed by the social theory of human capability, the study collected primary qualitative data from five NTD endemic counties of Kenya. The research interviewed key informants (n=21) involved in NTD activities and focus groups (n=5) consisting of 7-8 persons, infected or affected by NTDs. The main findings of the research indicate that despite the ongoing control strategies taking place in Kenya as outlined by the World Health Organization (WHO), universal health care and community support groups for persons suffering from impairment and disfigurement as a result of NTD infection is severely lacking. Thus this research recommends the establishment of support and counselling services for people suffering from NTDs that cause long term disability such as leprosy, lymphatic filariasis, trachoma and snake bites to provide psychosocial support and other coping mechanisms that improve their wellbeing. Also, the research recommends the provision of universal healthcare to cover the diagnosis and treatment of NTDs in Kenya at an affordable or no costs to all citizens. Hence, this research suggests that NTD stakeholders embrace human agency in the provision of context specific solutions to NTD control programs.

1648

MATHEMATICAL MODELING OF THE INTERRUPTION OF THE TRANSMISSION OF SOIL TRANSMITTED HELMINTHS INFECTIONS IN KENYA

Collins Okoyo[1], Graham Medley[2], Charles Mwandawiro[1], Nelson Owuor[3]

[1]*Kenya Medical Research Institute, Nairobi, Kenya,* [2]*London School of Hygiene & Tropical Medicine, London, United Kingdom,* [3]*University of Nairobi, Nairobi, Kenya*

Kenya, has been conducting regular treatment program for the last five years among school aged children as a way to reduce soil-transmitted helminths (STH) infections burden in the country. However, the point of interruption of transmission of these infections still remains unclear. We analyzed an age structured mathematical model to predict the point of interruption of these infections in Kenya. Objective was to develop and analyze an age structured model of the STH population dynamics under a regular STH treatment program to determine infection transmission rate, the point of infection interruption, and the optimal interpulse treatment interval sufficient to achieve STH infections elimination in Kenya. The model was applied to three age groups: preschool age children (2 to 4 years), school age children (5 to 14 years) and adults (above 14 years) and investigated the potential for STH elimination with finite rounds of treatment while allowing the STH distribution to change dynamically as a function of treatment frequency and treatment coverage. The model was verified using a five year field data from the Kenyan National School Based Deworming Program (NSBDP) for all the three main types of STHs. The model behaviour demonstrated convincingly an accurate predictions of prevalence and mean intensities of infections during and after treatment rounds in each of the age groups. The model indicated that the benefit derived from the regular treatment increases non-linearly with the treatment rounds and coverage. Additionally, it depicted that for elimination to be achieved within a shorter time period in the general population and within each age group, higher treatment coverage and biannual treatment rounds are more effective. The model captured the dynamics of the STH burdens in key populations under regular treatment program as elimination is approached. It aided in examining the role of age structure to the persistent STH infections in Kenya. As a result of these findings, we aim to advise the STH control programs on the right mix of strategies needed to achieve faster elimination of the STH infections in Kenya.

1649

LOOKING AHEAD IN MALARIA: R21/MATRIX-M, AN EXCITING NEW VACCINE CANDIDATE

Mehreen S. Datoo[1], Meera Madhavan[1], Duncan Bellamy[1], Megan Baker[1], Fernando Ramos-Lopez[1], Amy Flaxman[1], Nick J. Edwards[1], Daniel Jenkin[1], Hazel Morrison[1], Rebecca Makinson[1], Jeremy Aboagye[1], Ian Poulton[1], Nguyen Tran[1], Alison Lawrie[1], Anna Goodman[2], Katrina Pollock[3], Andrew Blagborough[4], Jake Baum[5], Saul Faust[6], Brian Angus[7], Umesh Shaligram[8], Katie J. Ewer[1], Adrian V. Hill[1]

[1]*Jenner Institute, University of Oxford, Oxford, United Kingdom,* [2]*Department of Infectious Diseases, Guy's & St Thomas' NHS Foundation, London, United Kingdom,* [3]*NIHR Imperial Clinical Research Facility, Imperial College, London, United Kingdom,* [4]*University of Cambridge, Cambridge, United Kingdom,* [5]*Department of Life Sciences, Imperial College, London, United Kingdom,* [6]*NIHR Wellcome Trust Clinical Research Facility, University of Southampton, Southampton, United Kingdom,* [7]*Oxford University Hospitals NHS Foundation Trust, Oxford, United Kingdom,* [8]*Serum Institute of India, Pune, India*

Stalled progress in controlling malaria mortality in the most affected African countries highlights the urgent need for an effective deployable vaccine, manufactured at low cost, and at a scale of 200 million doses annually. We report on the safety, immunogenicity and efficacy of a new anti-sporozoite vaccine, R21, which has many advantages over the current lead candidate, RTS,S. R21 and RTS,S immunogens induce high titres of protective antibodies in pre-clinical studies. However, the greater coverage of the nanoparticle surface of R21 by the *P. falciparum* circumsporozoite (CS) antigen generates a more focused anti-malarial response. We conducted a controlled human malaria infection (CHMI) trial of R21 adjuvanted with Matrix-M (MM). While testing efficacy in the standard regime (3 doses, 4 weekly intervals), we aimed to test if immunogenicity and efficacy improved with a delayed 3rd dose. The delayed dose was given 6 months after the first. One month after the 3rd dose of R21/MM, volunteers underwent CHMI. 10 of 16 vaccinees (63%) were sterilely protected in the group using the standard regime, while 9 of 12 vaccinees (75%) were protected in the group with the delayed 3rd dose. IgG to NANP, the central repeat of *P. falciparum* CS protein, was induced in all vaccinees. Levels were significantly higher with a delayed 3rd dose prior to CHMI. Using 1/5th of the dose of immunogen employed by RTS,S, we demonstrate an improved safety profile, and evidence of better immunogenicity and efficacy, that may be enhanced further by delaying the 3rd dose. Additionally, we will report results using a delayed, fractional 3rd dose of R21/MM. We will also boost the previously protected volunteers with a 4th dose of R21/MM and assess durability of immune responses and efficacy through re-challenge. These data improve on the published efficacy of all other subunit malaria vaccines tested, leading to ongoing highly promising safety and efficacy trials of R21/MM in East & West Africa in the target population of infants and children. The future aim is rollout across endemic areas, significantly contributing to global efforts of malaria control and subsequently eradication.

1651

GENOME, PROTEOME, AND IMMUNONE DATA EXPLAIN WHY CONTROLLED HUMAN MALARIA INFECTION WITH SPOROZOITES OF THE PF7G8 CLONE OF *PLASMODIUM FALCIPARUM* IS A RIGOROUS PREDICTOR OF THE EFFICACY OF THE PFNF54-BASED PFSPZ VACCINE IN AFRICA

Joana C. Silva[1], Ankit Dwivedi[1], Kara A. Moser[1], Mahamadou S. Sissoko[2], Judith E. Epstein[3], Sara Healy[4], Kirsten E. Lyke[1], Benjamin Mordmueller[5], Tao Li[6], Tooba Murshedkar[6], Peter Kremsner[5], Patrick E. Duffy[4], Thomas Richie[6], B. Kim Lee Sim[6], Stephen L. Hoffman[6]

[1]*University of Maryland School of Medicine, Baltimore, MD, United States,* [2]*University of Science, Techniques and Technologies of Bamako, Bamako, Mali,* [3]*Naval Medical Research Center, Silver Spring, MD, United States,* [4]*National Institutes of Health, Bethesda, MD, United States,* [5]*University of Tübingen, Tübingen, Germany,* [6]*Sanaria, Inc., Rockville, MD, United States*

More than 80% of the 1000s of annual cases of malaria in US and EU travelers are acquired in Africa, and >80% of these cases are caused by *Plasmodium falciparum* (Pf). To reduce this travel risk, Sanaria and colleagues are developing PfSPZ Vaccine, composed of aseptic, purified, cryopreserved Pf sporozoites (SPZ) of the African PfNF54 strain. Because of the difficulty powering and safely conducting a double blind, placebo-controlled field trial in travelers to assess vaccine efficacy (VE), we propose using controlled human malaria infection (CHMI) as a surrogate. Here, we argue that VE against CHMI using heterologous (different from vaccine strain) Pf parasites that are more divergent genetically and antigenically than any African parasite will provide a stringent predictor of VE in Africa. This proposal rests on two sets of data in which identical PfSPZ Vaccine regimens were tested against (1) heterologous CHMI using the Brazilian strain Pf7G8 in malaria-naïve adults in the US and (2) naturally transmitted Pf in malaria-exposed adults in Mali (who show markedly diminished immune responses to PfSPZ Vaccine, likely due to the immune regulation resulting from repeated episodes of parasitemia). Considering both cases, VE in the field over 24 weeks was as good as or better than against heterologous CHMI with 7G8 at 24 weeks. To explain this finding, we quantified genetic differences genome-wide and in the proteome and predicted CD8+ T cell epitopes of NF54 relative to 709 Pf isolates from East, West, and Central Africa and to Pf7G8, and found that Pf7G8 is more distant from PfNF54 than any African isolate. As CD8 T cell epitopes are the primary mechanism of protection, the reduced proportion of shared epitopes with Pf7G8 compared to African strains likely explains the stringency of 7G8 CHMI. Therefore, we posit that Pf7G8 CHMI is an appropriate surrogate for field efficacy in Africa, especially given that the immune responses induced by PfSPZ Vaccine in malaria-naïve adults are much stronger than in malaria-exposed adults. Heterologous CHMI with 7G8 should therefore provide pivotal data to support PfSPZ Vaccine licensure for travelers to Africa.

1652

A FOUR-TIERED HIGH-THROUGHPUT APPROACH IDENTIFIES TWO NOVEL TRANSMISSION BLOCKING VACCINE CANDIDATES WITH POTENT TRANSMISSION REDUCING ACTIVITY

Miranda S. Oakley[1], Nitin Verma[1], Abhai K. Tripathi[2], Hong Zheng[1], Edward Essuman[1], Ankit Puri[1], Richard A. Skelton[1], Scott Meredith[1], Kazuyo Takeda[1], Victoria Majam[1], Godfree Mlambo[2], Kazutoyo Miura[3], Carole A. Long[3], Sanjai Kumar[1]

[1]*FDA, Silver Spring, MD, United States,* [2]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [3]*NIAID, Rockville, MD, United States*

Despite decades of research, there are only a few *Plasmodium falciparum* antigens that indisputably and reproducibly demonstrate transmission blocking immunity when tested as transmission blocking vaccine (TBV) candidates. However, some of these TBV candidates have been shown to produce systemic reactogenicity and short lasting antibody responses. In this study, we used a four tiered high-throughput approach to identify and evaluate novel TBV candidates. First, using a genomics approach, we selected *P. falciparum* genes that are abundantly expressed in the gametocyte stage of the parasite for evaluation as novel TBV candidates. Second, we expressed these candidate genes as recombinant proteins. Third, we vaccinated mice with 16 recombinant proteins in two different adjuvant formulations to produce antisera. Lastly, we tested the efficacy of raised sera by measuring transmission reducing activity (TRA) in the Standard Membrane Feeding Assay (SMFA). Using this approach, we identified two novel TBV candidates that displayed 93% and 84% TRA activity at 750 ug/mL, respectively. In depth studies were performed to characterize the genetic diversity of, stage specific expression by, and natural immunity to these two molecules to evaluate their suitability as TBV candidates. In summary, we have identified two promising TBV

candidates that display limited polymorphism in 218 parasite isolates, are pan-developmentally expressed in the sporozoite, blood, and gametocyte stages of the parasite as shown by qPCR and confocal microscopy studies, and induce natural immunity that could boost vaccine-induced immunity as demonstrated by ELISA in a population of Ghanaian adults. The detailed biological characterization and transmission blocking efficacy of these antigens will be presented.

1653

STRUCTURAL DELINEATION OF NEUTRALIZING EPITOPE ON MALARIA ANTIGEN PFS230D1

Wai Kwan Tang[1], Camila H. Coelho[1], Martin Burkhardt[1], Olga Muratova[1], Nichole D. Salinas[1], Reiter Karine[1], MacDonald J. Nicholas[1], Nguyen Vu[1], Herrera Raul[1], Richard Shimp[1], David L. Narum[1], Jacob D. Galson[2], Thiago Luiz Alves E Silva[1], Miranda Byrne-Steele[3], Wenjing Pan[3], Xiaohong Hou[3], Brittany Brown[3], Mary Eisenhower[3], Jian Han[3], Joel Vega-Rodriguez[1], Johannes Truck[4], Justin J. Taylor[5], Issaka Sagara[6], Jonathan P. Renn[1], Duffy E. Patrick[1], Niraj H. Tolia[1]

[1]*National Institutes of Health, Bethesda, MD, United States,* [2]*University children's hospital, Zurich, Switzerland,* [3]*iRepertoire Inc, Huntsville, AL, United States,* [4]*University of Zurich, Zurich, Switzerland,* [5]*Fred Hutchinson Cancer Research Center, Seattle, WA, United States,* [6]*University of Sciences, Bamako, Mali*

Blocking the transmission of *Plasmodium falciparum* from mosquitoes to human host is one way to eliminate malaria. Pfs230 is a protein that presents on the surface of gamete, plays an important role in sexual-stage development of the parasite. Vaccines that induce antibodies targeting Pfs230 may therefore halt the transmission of the parasites. Here, we identify a human monoclonal antibody (mAb) LMIV230-01 from Malian adults vaccinated against Pfs230 domain 1 (Pfs230D1). The mAb interacts with both full-length Pfs230 and Pfs230D1, and potently blocked transmission to mosquitoes. Structure determination of Pfs230D1 in complex with LMIV230-01 reveals a 6-Cys domain fold of Pfs230D1. We identify a large neutralizing epitope of LMIV230-01 on Pfs230D1 which comprises six secondary structural elements. The complex is held by both hydrophobic interactions and hydrogen bonds, showing a nanomolar range affinity. Analysis of Pfs230D1 sequences reveals that polymorphisms are low for residues involved at the binding interface. Further mutagenesis study shows these polymorphic variants can all bind to LMIV230-01 and is strain transcendent. Our data indicate that immunization with Pfs230D1 produces potent neutralizing antibody that engage a large and highly conserved conformational exposed epitope. This study provides a rational basis to improve vaccines and develop therapeutic antibodies for malaria elimination.

1654

STRUCTURAL BASIS FOR PLACENTAL SEQUESTRATION OF *PLASMODIUM FALCIPARUM* BY VAR2CSA

Rui Ma, Tengfei Lian, Rick Huang, Jonathan P. Renn, Jennifer D. Petersen, Joshua Zimmerberg, Jiansen Jiang, Patrick E. Duffy, Niraj H. Tolia

National Institute of Health, Bethesda, MD, United States

Placental malaria is caused by the accumulation of *Plasmodium falciparum* parasites in the placenta resulting in poor pregnancy outcomes and mortality for mothers and their offspring. Parasite sequestration is mediated by binding of the parasite protein VAR2CSA to its receptor chondroitin sulfate A (CSA) on the surface of the syncytiotrophoblast. VAR2CSA is a 350 kDa polymorphic multi-domain protein of the PfEMP1 variant surface antigen family and is the leading vaccine candidate to prevent placental malaria. Here, we determined the atomic-resolution structure of the full-length ectodomain of VAR2CSA from *P. falciparum* strain NF54 in complex with CSA, and VAR2CSA from *P. falciparum* strain FCR3 by cryo-electron microscopy. This study provides structural definition for a full-length PfEMP-1 family member. Six Duffy-binding like (DBL) domains and two Interdomain (ID) regions interact in an interwoven manner to stabilize VAR2CSA. The structures resemble the number 7 with a stable core flanked by a flexible arm. CSA traverses the core domain by binding within channels in the core. The CSA binding elements are conserved across VAR2CSA variants and are flanked by polymorphic segments suggesting immune selection outside the CSA-binding sites. This work defines conserved and polymorphic regions of VAR2CSA with direct applicability for developing a strain-transcending vaccine that focuses the immune response to conserved segments. Receptor-free and receptor-bound VAR2CSA are structurally similar indicating no major domain rearrangements are required to bind CSA. This work rationalizes a body of data and establishes a path for the structure-guided design of protective VAR2CSA vaccines and therapeutics.

1655

IMMUNOFOCUSING THE HUMORAL RESPONSE TO FUNCTIONAL EPITOPES OF THE ANAPN1 MALARIA TRANSMISSION-BLOCKING VACCINE ANTIGEN POTENTIATES EFFICACY

Nicole Bender[1], Prachi Khare[1], Juan Martinez[2], Rebecca Tweedel[1], Vincent Nyasembe[1], Abhai Tripathi[3], Dustin Miller[4], Timothy Hamerly[1], Eric Vela[2], Ronald Cobb[2], Matthias Harbers[5], Rhoel Dinglasan[1]

[1]*University of Florida, Gainesville, FL, United States,* [2]*Ology Bioservices Inc, Alachua, FL, United States,* [3]*Johns Hopkins University, Baltimore, MD, United States,* [4]*Centers for Disease Control, Atlanta, GA, United States,* [5]*CellFree Sciences Co., Ltd., Yokohama, Japan*

Malaria is a devastating vector-borne disease caused by protozoan parasites of the genus *Plasmodium*, which causes roughly 435,000 deaths a year, predominantly children under the age of five in sub-Saharan Africa. Malarial parasites have an obligatory developmental cycle in the *Anopheles* mosquito vector. Malaria elimination has stalled, and new tools needed more than ever to prevent residual transmission. Transmission-blocking vaccines (TBVs) work by preventing sporogony in the mosquito vector, thereby blocking human-mosquito-human transmission. We have shown that antibodies against the mosquito midgut surface protein Anopheline Alanyl aminopeptidase N (AnAPN1) block *Plasmodium* parasites from traversing the mosquito gut epithelium, effectively preventing the cascade of secondary infections. By solving the crystal structure of AnAPN1, two important transmission-blocking (T-B) epitopes (peptides 7 and 9) and an immune-decoy epitope (peptide 1) were identified. We hypothesized that outbred mice generate a disproportionate antibody response to peptide 1; thereby reducing the overall T-B activity of immune sera. To immunofocus the vertebrate humoral response to these two key epitopes and thereby increase T-B activity, we developed a new AnAPN1 construct, UF6, lacking the decoy epitope and containing two copies of the critical T-B epitopes. We evaluated this new AnAPN1 immunogen with a human safe adjuvant, Glucopyranosyl Lipid Adjuvant in a liposomal formulation with saponin QS21 (GLA-LSQ), in outbred CD1 mice and cynomolgus macaques. Through a battery of immunological and functional assays we found the new construct to be immunogenic and capable of immunofocusing the immune response to peptide 9, one of the key T-B epitopes, resulting in potent T-B activity at lower concentrations in mosquito feeding studies.

1656

LEISHMANIA-INFECTED MACROPHAGES RELEASE EXTRACELLULAR VESICLES THAT ACTIVATE ENDOTHELIAL CELL PROCESSES AND MAY PROMOTE VASCULARIZATION OF *LEISHMANIA* LESIONS

Anna E. Gioseffi[1], Kha Van[2], Keily Ortega[2], Phil Yates[2], Peter Kima[2]

[A]*Microbiology and Cell Science,* [1]*University of Florida, Gainesville, FL, United States*

Leishmania donovani is an intracellular eukaryotic parasite that causes visceral leishmaniasis, infecting 0.5 million new cases globally per year.

To better understand the pathogenesis of *L. donovani*, we characterized the infection-derived proteins that are released in extracellular vesicles (EVs) from infected cells (LieEVs). We had previously shown that LieEVs were composed of host and parasite derived molecules that are specific to infection. Pathway analysis of host molecules in LieEVs revealed that they are composed of molecules that have the capacity to promote vascular changes in tissues. This led us to hypothesize that *Leishmania*-infected cells release EVs that can promote vascular changes of *Leishmania* lesions, which has been shown to be important for lesion development *in vivo*. In addition to host derived molecules, we also identified parasite derived molecules in LieEVs, including LdVash, a putative parasite homolog of mammalian Vasohibins. Using surrogate *in vitro* assays of angiogenesis, we found that LieEVs induced Human umbilical vein endothelial cells (HUVECs) to release angiogenesis promoting cytokines and chemokines, including VEGF-A, IL-8 and G-CSF/CSF-3. Moreover, intact LieEVs promoted higher levels of epithelial cell migration and tube formation by HUVECs compared to disrupted LieEVs or EVs from uninfected macrophages (CeEVs), which suggested the importance of vesicle structural integrity for their functions. In additional studies, parasites expressing LdVash tagged with mNeonGreen (LdVash/mNG) were used to study the intracellular trafficking of LdVash in infected cells to understand the biogenesis of LieEVs. Taken together, we provide evidence that *Leishmania*-infected macrophages release EVs that contain host and parasite derived molecules, including a *Leishmania* homolog of Vasohibin, which may contribute to vascular changes of the parasite lesion.

1657

EFFECTOR FUNCTION PRIOR TO ESTABLISHMENT OF THE PHAGOSOMAL PATHOGEN NICHE IS REQUIRED FOR PROTECTIVE CD4+ T CELL-MEDIATED IMMUNITY AGAINST LEISHMANIA

Leah Hohman, Matheus B.H. Carneiro, Rachel Kratofil, Nathan C. Peters

Snyder Institute for Chronic Diseases, Department of Immunology, University of Calgary, Calgary, AB, Canada

Leishmania represents an appealing model organism to study CD4+T cell-mediated protective immunity against phagosomal pathogens and features localized primary and secondary infection sites with defined innate and adaptive responses. Upon secondary challenge of chronic L. major-infected C57Bl/6 mice, rapid delivery of CD4+T effector (TEFF) function via IFN-g-mediated activation of infected monocytes is associated with optimal immunity. However, the absolute requirement for immediate effector function has yet to be demonstrated. Thus, we isolated time as a variable in the delivery of Ly6C+CD4+TEFF-mediated effector function. We adoptively transferred (AT) chronic mouse-derived Ly6C+CD4+TEFFs into naïve recipients immediately (D0) or 4 days (D4) post-L. major challenge. In this time window Leishmania establishes an intracellular niche but does not proliferate. At day 21 post-challenge, D4 AT resulted in a total loss of the parasite control mediated by D0 transfer. Dose titration of Leishmania-specific Th1 TcR-Tg T cells revealed that no number of TcR-Tg Th1 cells transferred at D4 overcame the requirement for rapid D0 immunity. To address whether parasite niche establishment modulated Th1 TEFF cell recruitment, intravital imaging was employed. Ag-sp T cells were present in the infected dermis at significantly lower numbers following D4 vs D0 transfer at 4 days post-T cell transfer, indicating a recruitment deficit. Additionally, in-vitro co-culture of Ag-sp Th1 TEFFs with infected monocytes either immediately (D0) or 2 days (D2) after infection revealed parasite killing and NOS-2 production were significantly impaired in the D2 group relative to D0. Collectively, these findings suggest that in the absence of immediate CD4+T cell immunity, parasite niche establishment in host phagocytes inhibits both Th1 cell recruitment and parasite killing. We propose that near-immediate effector function mediated by circulating TEFF cells is required to prevent immunomodulation of permissive monocytes by Leishmania and represents an important consideration for prophylactic vaccination against phagosomal pathogens.

1658

THE ROLE OF THE GPI ANCHOR IN IMMUNITY TO *TOXOPLASMA GONDII*

Julia Alvarez[1], Scott P Souza[1], Elisabet Gas-Pascual[2], Jessica N Wilson[1], Safuwra Wizzard[1], Brooke Wilson[1], Chistopher M West[2], Kirk DC Jensen[1]

[1]*University of California Merced, Merced, CA, United States,* [2]*University of Georgia, Athens, GA, United States*

The development of an effective vaccine against parasitic infections like *Toxoplasma gondii* requires more understanding about the battle between host and pathogen. What allows for some mouse strains to survive while others succumb? What antigens elicit the strongest antibody response? Utilizing a genetic screen of recombinant inbred mice, we discovered a gene that correlates with survival to secondary infections with virulent strains of *T. gondii*. This gene, *Nfkbid*, is an atypical regulator of NFκB and mice lacking *Nfkbid* fail to generate parasite-specific antibodies and do not survive secondary infections with *T. gondii*. We hypothesized that structural features of parasic antigens may necessitate *Nfκbid*-dependent immunity to *T. gondii*. Glycophosphatidylinositol lipids (GIPLs) cover the surface of most parasites and can attach surface antigens to the plasma membrane. *T. gondii* GIPLs are known to be highly immunogenic for IgM antibodies and are recognized by Toll-like receptors. Here we report that antibody reactivity to known GPI-anchored proteins of *T. gondii* is lost after cleavage of the GPI lipid moiety with PI-PLC treatment. Further, we have created *T. gondii* mutants of a previously uncharacterized glycotransferase, responsible for the addition of an immunogenic n-acetylgalactose (+/- glucose) side-chain to the GPI backbone. These mutants show dramatic loss of IgM reactivity to their GIPL when probed with chronic sera and exhibit increased virulence during primary and secondary infections. Since *Nfkbid* is downstream of TLR signaling and *T. gondii* GPI triggers TLR2 and TLR4, we hypothesized TLR2/4 signaling may be required for antibody-mediated immunity to *T. gondii*. Consistent with this supposition, vaccination does not protect Tlr2-/- mice from *T. gondii* challenge and this correlates with reduced parasite-specific IgG1 responses. The model we are entertaining is that cell autonomous TLR-driven B cell responses are required for immunity and antibody reactivity to GPI-moieties of *T. gondii*.

1659

A NOVEL PROTEIN COMPLEX IS ESSENTIAL FOR THE MATURATION OF TRANSMISSION-STAGE MALARIA PARASITES

Rebecca Clements[1], Esrah W. Du[2], James P. McGee[2], Vincent Streva[2], Jeffrey D. Dvorin[2]

[1]*Biological and Biomedical Sciences, Harvard Medical School, Boston, MA, United States,* [2]*Division of Infectious Diseases, Boston Children's Hospital, Boston, MA, United States*

Human malaria, which is caused by Plasmodium parasites, remains an important cause of global morbidity and mortality. To successfully generate new antimalarials, we must gain a better understanding of the fundamental cell biology of *Plasmodium falciparum*, the parasite responsible for the deadliest cases of malaria. A membranous scaffold and group of associated proteins called the inner membrane complex (IMC) serves as a structural support during major morphological changes throughout the life cycle of *P. falciparum*, including segmentation of daughter cells during asexual replication and formation of transmission-stage parasites via gametocytogenesis. The basal complex lines the emerging edge of the IMC during segmentation and is likely critical for expansion of the IMC. It is unknown, however, what drives expansion of the IMC during gametocytogenesis. Here we describe the discovery of a novel basal complex protein, PfBLEB (Baso-Lateral Expansion Boundary), PF3D7_0704300. Although PfBLEB expression is not necessary for asexual replication *in vitro*, we find that PfBLEB is essential for gametocyte formation. Parasites lacking PfBLEB harbor defects in IMC expansion and are unable to form mature, transmissible gametocytes. We demonstrate expression of PfBLEB throughout gametocytogenesis, and find that PfBLEB

is part of a novel protein complex in gametocytes, which we name the gametocyte lateral complex. The gametocyte lateral complex is distinct in composition from the asexual basal complex, but similarly localizes to the expanding edge of the IMC. This study is the first demonstration of a role for a basal complex protein outside of asexual division, and, importantly, highlights a potential molecular target for ablation of malaria transmission.

1660

SHIFTING PERSPECTIVES: A MODIFICATION TO THE LIFE CYCLE OF TRYPANOSOMA BRUCEI

Jaime Lisack, Sarah Schuster, Ines Subota, Markus Engstler

Universität Würzburg, Bavaria, Germany

African trypanosomes are the causative agent of Human African Trypanosomiasis (HAT) and thecattle plague, Nagana. As with all vector-borne diseases, transmission is intimately tied toparasite survival and propagation in the vector, the blood-sucking tsetse fly. Two main stages ofT. brucei live in the mammalian host, the proliferative long slender form and the cell cyclearrested short stumpy stage. The transition from slender trypanosomes into stumpy occurs via aquorum sensing mechanism, mediated by the parasite-excreted stumpy induction factor (SIF). Asslender populations grow, the SIF threshold is reached and stumpy trypanosomes form. Asidefrom morphological and metabolic changes, stumpy trypanosomes also express the proteinassociated with differentiation 1 (PAD1) (Matthews, 2009). The switch from slender to stumpytrypanosomes is thought to accomplish two things. First, it auto-regulates parasite density andhence, prolongs survival of the host. Second, stumpy forms are thought to be 'pre-adapted' tosurvival in the tsetse fly vector. It has long been believed that upon uptake from the mammalianblood, only the 'pre-adapted' stumpy trypanosomes can survive in the fly midgut, while slendertrypanosomes were thought to die. Keeping slender trypanosome populations below the SIFthreshold and diluting parasites at different densities for in vivo fly infections, we show that bothslender and stumpy trypanosomes can propagate with comparable rates in the tsetse fly. Weamassed a large dataset of fly infections and dynamics, further showing that that only onetrypanosome, slender or stumpy, is necessary to infect a tsetse fly. Next, we looked atdifferentiation hallmarks at the early stages of differentiation, both in cell culture and in the fly.Here, we found that upon differentiation, PAD1, thought to indicate stumpy formation in themammalian host, is expressed during slender trypanosome differentiation in the fly midgut,without cell cycle arrest or morphological transition to the stumpy stage. Thus, both stumpy andslender cells can complete the life and transmission cycle inside the tsetse fly vector. Theseresults not only hold implications regarding the life cycle of T. brucei but also on transmissiondynamics. This data could help answer the long-held question of how disease incidence can besustained in chronic mammalian infections, at low blood parasitemia, where stumpytrypanosomes are characteristically absent.

1661

IMMUNOMODULATORY EFFECTS OF HELMINTH (LITOMOSOIDES SIGMODONTIS) ANTIGEN ON HUMAN BLOOD CELLS

Priscilla Kini, Priscilla Kyerewaa Okomeng, Alexander Kwarteng

Department of Biochemistry and Biotechnology, College of Science, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana

Helminth infections pose huge health burden to most endemic countries and are immunologically characterized by Th2 responses. The immune profile of helminth-infected individuals is believed to be compounded in settings where the likelihood of being co-infected with bacteria and viruses is high. While bacterial and viral infections are characterized with Th1 responses, however, the immune profiles of co-infected individuals in helminth-endemic regions remain underexplored. In this study, we stimulated whole blood from healthy adult participants (n=9) in an in vitro setting with Litimosoides sigmodontis (LS) antigen, staphylococcal enterotoxin B (SEB) and LS plus SEB antigens and cultured for 48 hr. Subsequently, the frequencies of cell surface markers (CD4 and CD69) and cytokines (IL-10 and IFN-γ) was measured using fluorescent activated cell sorter (FACS) and data analyzed using FlowJo software and GraphPad Prism (version 6). Our results show that there was no significant difference in the expression of activated CD4+ T cells, IL-10 and IFN-γ levels between LS activated cells and the unstimulated control. Interestingly, SEB stimulation led to a significantly higher frequency of CD4+-producing-IL-10+ T cells but significantly reduced CD4+ producing IFN-γ+ T cells. Of note, LS plus SEB stimulations mirrored that of SEB stimulation alone but the trend was slightly higher for the investigated immune markers and cytokines. Therefore, these data provide strong evidence that in developing vaccines for helminth infections, extensive co-infection in vitro studies are crucial given the possible immunomodulatory potentials on fate decisions of the cells during cell-mediated and humoral responses.

1662

DETERMINANTS OF PRAZIQUANTEL MASS DRUG ADMINISTRATION FAILURE TO CONTROL SCHISTOSOMIASIS INFECTION IN A PERSISTENT FOCI OF TRANSMISSION: A CROSS SECTIONAL STUDY OF FIVE DIFFERENTIALLY AFFECTED NEIGHBOURING VILLAGES IN RURAL CAMEROON

Donald Severin Kamdem[1], Erve Martial Kuemkon[2], Francis Konhawa[2], Leonel Meyo Kamguia[2], Gladys K. Tchanana[2], Frungwa Nche[2], Alim Oumarou[2], Essomba Rene Ghislain[2], Marie Claire Okomo Assoumou[2], Frank Brombacher[1], Justin Komguep Nono[1]

[1]International Centre for Genetic Engineering and Biotechnology, Cape Town Component, Cape Town, South Africa, [2]Ministry of Public Health, Yaounde, Cameroon

Background: Schistosomiasis remains a worldwide serious public health burden threatening millions of people living in areas with poor sanitation. Although mass drug administration of Praziquantel (PZQ) is commonly used to control the disease, the re-infection rate remains very high in some endemic areas. This study therefore assessed and reported all determinants of susceptibility/resistance to Schistosoma mansoni (Sm) infection in five differentially affected neighbouring villages (Ediolomo; Kedia; Bongando; Yoro 1 and Yoro 2) in the Bokito subdivision in rural Cameroon.Methods: A cross-sectional study including overall 1,002 consented school children was conducted (127 school children from Ediolomo; 189 from Kedia; 299 from Bongando; 221 from Yoro 1 and 166 from Yoro 2). Enrolled school children were screened for Sm eggs using Kato katz technique and all statistics were performed using the software R at 95% confidence. Finally, the cartography of the study area was established using the ArcGIS 10.2 software.Results: Despite belonging to the same endemic area, we found a great discrepancy of Sm prevalence among the 5 neighbouring villages from 1.6% to 39.7%. Yoro 1 (OR 12.57; 95% CI 2.97 - 53.07) and Yoro 2 (OR 41.24; 95% CI 9.86 - 172.49) villages, the sites of highest prevalence were respectively found around 13-fold and 42-fold at higher risk of infection ($p < 0.05$). At the village level, whereas the availability of boreholes was clearly a determinant of resistance to the infection ($p < 0.0001$), the proximity to the river ($p < 0.0001$), the frequency of contact with water ($p = 0.05$), the length of residence in the endemic area ($p = 0.03$), the number of previous PZQ treatment ($p = 0.03$), the number of persons living in the same house ($p = 0.0001$) as well as the compliance to PZQ treatment ($p = 0.05$) were determinants of susceptibility to Sm infection. At the individual level, the BMI was significantly associated with Sm infection (AOR 2.65; 95% CI 1.19 - 5.93). Moreover, a higher Body Mass Index ($p = 0.01$), and a higher rate of sub-therapeutic PZQ treatment ($p = 0.02$) was found in Sm positive participants. Likewise, a higher BMI was also associated with a higher proportion of sub-therapeutic treatment in our study populations ($p < 0.0001$).Conclusion: Altogether, our observations point at the deworming strategy based on the participants body height as a major limitation of ongoing MDA success in some of these sites. This height-based approach of treatment (dose-pole) might lead to a sub-dosage of the drug to incompletely clear the worm and support the persistence of the infection. Therefore, PZQ treatment based

on body weight could circumvent this and help foster disease elimination in schistosomiasis-endemic and operationally failing sites in rural Cameroon.

1663

HIGH-THROUGHPUT FUNCTIONALIZATION OF THE TOXOPLASMA *GONDII* PROTEOME

Tyler Smith[1], Gabriella Lopez-Perez[2], Emily Shortt[1], Sebastian Lourido[1]

[1]Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology, Cambridge, MA, United States, [2]University of Puerto Rico at Mayagüez, Mayagüez, PR, United States

Apicomplexans are some of nature's most widespread parasites and include the causative agents of toxoplasmosis (*Toxoplasma gondii*), cryptosporidiosis (*Cryptosporidium* spp.), and malaria (*Plasmodium* spp.). These parasites have evolved an array of phylum-specific adaptations; however, most apicomplexan proteins have not been functionally characterized. Recently developed CRISPR-Cas9 screening platforms enable the high-throughput characterization of the *T. gondii* genome but lack temporal control and require follow-up studies to assign proteins to specific compartments and cellular pathways. We developed a high-throughput (HiT) CRISPR-mediated tagging vector to rapidly functionalize the C termini of target proteins with a synthetic sequence encoding protein tags or other regulatory elements. Utilizing the HiT vector, we tagged a library of 155 genes with the mini auxin-inducible degron (mAID) linked to a fluorophore and epitope tag. This enabled rapid and reversible knock-down of the targeted proteins. We assayed the function of each tagged mutant using pooled screens in the presence or absence of auxin. After subcloning the population we screened 1,160 arrayed clones by both lytic assay and microscopy via replica plating. In addition to assigning protein localizations, we were able to place clones within 7 unique phenotype profiles, encompassing a diversity of parasite biology. This system extends the applications of genome-wide screens into complex cellular phenotypes, providing a new versatile platform for the dissection of apicomplexan cell biology.

1664

THE *CRYPTOSPORIDIUM* SINGLE-CELL ATLAS REVEALS KEY LIFE CYCLE STAGES AND A COMMITMENT TO MALE AND FEMALE DEVELOPMENT

Katelyn A. Walzer, Jayesh Tandel, Jodi A. Gullicksrud, Stephen Carro, Eoin Whelan, Elise Krespan, Daniel P. Beiting, Boris Striepen

University of Pennsylvania, Philadelphia, PA, United States

The apicomplexan parasite *Cryptosporidium* is a leading global cause of diarrheal disease and infects millions of people each year, with a particularly high prevalence in south Asia and sub-Saharan Africa. The current treatment, nitazoxanide, is ineffective in immunocompromised patients and malnourished children, and there is no vaccine. Therefore, a great need exists for new and more effective therapeutics against *Cryptosporidium*. Transmission of the parasite occurs via the fecal-oral route, and the entire life cycle takes place in a single host: asexual growth, replication, and division take place in intestinal epithelial cells, followed by transition to a male or female form and sexual reproduction. Yet while a few molecular markers have been identified to demarcate this life cycle progression, the signaling pathways and gene expression changes involved in development remain largely unknown. Here, we used single-cell RNA sequencing of infected cultures and mice to determine the complete life cycle transcriptome of *Cryptosporidium in vitro* and *in vivo*. Analysis of 9,310 individual parasite transcriptomes revealed clear asexual cycle progression with an abrupt switch to either male or female development during the trophozoite stage. We find no transcriptional evidence for a type II meront, as gene expression dramatically changes only when transition to male or female occurs and none is noted in the prior asexual cycle. In asexual parasites, gene expression was driven by cell cycle progression dominated early by ribosomal biogenesis, processing, and assembly followed by protein folding and then DNA replication. Later asexual stages expressed many secreted proteins, including an abrupt transition to invasion related organelles. While females arrest *in vitro*, *in vivo* they progress to sporogony, and sporozoite and merozoite gene expression is highly similar. Importantly, single-cell transcriptional profiling revealed stage-specific and sex-specific expression of AP2 and Myb transcription factors, including distinct expression in early males, outlining a pathway for sex-specific commitment. Future work will focus on determining the functional roles of these transcriptional regulators. Overall, our work provides the first comprehensive view of *Cryptosporidium* gene expression over the entire life cycle and identifies the key genes in replicative, invasive, and sexual stages and the regulatory networks that control them.

1665

THE VSG-EXCLUSION (VEX) COMPLEX ORCHESTRATES VSG ALLELE-EXCLUSIVE INTERACTIONS WITH THE SPLICED-LEADER LOCUS IN TRYPANOSOMES

Joana Correia Faria[1], Vanessa Luzak[2], Laura S.M. Müller[2], Benedikt G. Brink[2], Sebastian Hutchinson[1], Lucy Glover[1], T. Nicolai Siegel[2], David Horn[1]

[1]The Wellcome Trust Centre for Anti-Infectives Research, School of Life Sciences, University of Dund, Dundee, United Kingdom, [2]Department of Veterinary Sciences, Experimental Parasitology, Ludwig-Maximilians-Universität München, Planegg-Martinsried, Germany

Trypanosoma brucei lacks classical enhancer sequences or regulated transcription initiation. However, it employs an enigmatic mechanism of monogenic antigen transcription to evade the host immune response. The association of one of approximately fifteen telomeric variant surface glycoprotein (VSG) genes with an RNA-polymerase-I (pol-I) transcription factory facilitates singular VSG expression. Notably, efficient RNA processing and maturation are somehow restricted to the active-VSG, suggesting that the access to RNA processing factors or substrates might be limiting. We recently identified a chromatin-associated VSG exclusion (VEX) complex containing VEX1 and VEX2; VEX2, in particular, is required to sustain VSG monogenic expression, but by an unknown mechanism (Faria et al, 2019; PMID: 31289266). We now show that the VEX-complex sustains allelic exclusion by co-ordinating allele-exclusive inter-chromosomal interactions with an RNA maturation locus. VEX1 or VEX2 ChIP-Seq analyses revealed an association not only with the single active-VSG but also with the spliced-leader (SL) array, a genomic locus that encodes for the SL-RNA, the key substrate for *trans*-splicing. Hi-C, DNA FISH and super-resolution microscopy revealed that only the active-VSG expression site (not the 'silent') was in close spatial proximity to the splicing locus in the 3D nuclear space; the association was dynamic during S phase but stably propagated through the cell cycle. Super-resolution microscopy also showed that VEX1 and VEX2 occupy the splicing and VSG expression site compartments, respectively. To further investigate the role of the VEX-complex, we tracked VSG expression sites, SL-arrays and pol-I. Following VEX2 knockdown, the pol-I compartment separates from the splicing compartment and disperses, while previously 'silent' VSG expression sites cluster around the SL-arrays and are derepressed. Therefore, VEX2 emerges as an exclusion factor that allows only one VSG to access the splicing compartment at a time. We found VEX2 to be a large (>200 kDa) multimeric RNA-helicase that forms a native complex of approximately 1 MDa. Notably, a family of helicases were recently shown to be global regulators of RNA-containing, phase-separated sub-nuclear organelles. Indeed, the VEX2 compartment is specifically disrupted following treatment with 1,6-hexanediol, which suppresses liquid-liquid phase-separation. We observe similar disruption of the VEX2 compartment following inhibition of transcription or splicing, suggesting that RNAs from the active-VSG expression site are required for the formation of VEX2 condensates. Our results reveal a novel VEX2-dependent mechanism that ensures both monogenic transcription and efficient RNA processing through the spatial integration of antigen transcription and mRNA splicing in a dedicated compartment.

Abstract Author Index

The number(s) following author name refers to the abstract number.

A

Abstract Author Index

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

B

Abstract Author Index

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

The number(s) following author name refers to the abstract number.

C

Abstract 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

Abstract Author Index

The number(s) following author name refers to the abstract number.

The number(s) following author name refers to the abstract number.

E

Abstract Author Index

The number(s) following author name refers to the abstract number.

F

The number(s) following author name refers to the abstract number.

G

Abstract Author Index

The number(s) following author name refers to the abstract number.

The number(s) following author name refers to the abstract number.

H

Abstract Author Index

The number(s) following author name refers to the abstract number.

I

The number(s) following author name refers to the abstract number.

J

Abstract Author Index

The number(s) following author name refers to the abstract number.

K

The number(s) following author name refers to the abstract number.

Abstract 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.

Abstract Author Index

The number(s) following author name refers to the abstract number.

M

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

N

The number(s) following author name refers to the abstract number.

Abstract 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

Abstract Author Index

The number(s) following author name refers to the abstract number.

The number(s) following author name refers to the abstract number.

Q

R

Abstract Author Index

The number(s) following author name refers to the abstract number.

S

Abstract Author Index

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

T

The number(s) following author name refers to the abstract number.

U

Abstract Author Index

The number(s) following author name refers to the abstract number.

V

W

The number(s) following author name refers to the abstract number.

Abstract Author Index

The number(s) following author name refers to the abstract number.

X

Y

Z

Abstract Author Index

The number(s) following author name refers to the abstract number.