999

AN *EX VIVO* MODEL FOR STUDYING THE EARLIEST PHASE OF HEPATIC SCHISTOSOMIASIS

Geoffrey Gobert

Queensland Institute of Medical Research, Brisbane, Australia

To investigate the earliest hepatic events associated with the deposition of schistosome eggs, we have established a novel technique, involving the culturing of naïve murine thin liver slices (250 µm) in conjunction with exposure to soluble egg antigens. This system has allowed us to identify the transcriptional events that contribute to the initiation of the subsequent granulomatous response. Initially tissue, without parasite antigen exposure, was analysed for general histological changes, the presence of liver enzymes indicative of hepato-toxicity and finally RNA quality. All of these parameters indicated the fidelity of the tissue, and the sterility over 48 hour period were maintained. Next to build on these research tools, we have employed microarray analysis of the tissue with and without parasite egg antigen, in order to allow us to follow the dynamics of antigen presentation, inflammation and general hepatotoxicity, which represent the initial phases that will lead to pathology. Findings from this *ex vivo* approach, are currently being integrated with data from our previously *in vivo* whole organ studies with *Schistosoma japonicum*. We eventually aim to identify the contribution of hepatic and systemic immune cell types in the host transcriptional response to egg deposition and the resulting granuloma formation in host liver.

1000

SCHISTOSOMIASIS JAPONICA DURING PREGNANCY IS ASSOCIATED WITH ELEVATED ENDOTOXIN LEVELS IN MATERNAL AND PLACENTAL COMPARTMENTS

Emily A. McDonald[1], Sunthorn Pond-Tor[1], Blanca Jarilla[2], Marianne Saglib[2], Analisa Gonzal[2], Remigio Olveda[2], Luz Acosta[2], Fusun Gundogan[3], Lisa M. Ganley-Leal[1], Jonathan D. Kurtis[1], Jennifer F. Friedman[1]

[1]Rhode Island Hospital, Providence, RI, United States, [2]Research Institute of Tropical Medicine, Manila, Philippines, [3]Women and Infants Hospital, Providence, RI, United States

Schistosomiasis affects approximately 40 million women of reproductive age, and chronic infection has been linked to elevated levels of endotoxin in circulation. Whether this is also true in pregnancy complicated with schistosomiasis has not been evaluated. In this study, we measured endotoxin levels in maternal peripheral (32 wks gestation), placental and newborn plasma collected from a cohort of 133 women in Leyte, The Philippines. Birth outcomes, cord blood, placental biopsy and placental blood were collected at delivery; endotoxin levels were measured in all plasma samples. Placental biopsies were evaluated for a number of histopathological outcomes related to placental inflammation. After adjusting for confounders, endotoxin levels in pregnant women with schistosomiasis were higher in the maternal and placental plasma than in uninfected women (1.3-fold, $P = 0.03$ and 2.4-fold, $P < 0.001$, respectively). Premature birth and acute chorioamnionitis were associated with elevated levels of endotoxin in the placental plasma (2.5-fold higher endotoxin in premature births, $P = 0.01$, 2.0-fold in acute chorioamnionitis, $P = 0.04$). A host of pro-inflammatory cytokines such as IL-6 (7.1-fold, $P < 0.001$), TNF-α (6.1-fold, $P <0.001$), IFN-γ (1.8-fold, P 0.05), IL-1 (14.7-fold, $P <0.001$), and CRP (2.8-fold, $P <0.001$) were elevated in maternal plasma among women with endotoxin levels in the highest tertile of the distribution. Additionally, some anti-inflammatory cytokines including IL-10 (2.1-fold, $P <0.001$), IL-5 (2.2-fold, $P =0.01$), and IL-13 (2.1-fold, $P <0.001$) were elevated in the placental plasma of these subjects. We have previously shown that schistosomiasis during pregnancy can elicit a pro-inflammatory cytokine response in placental, maternal and cord blood. Herein, we report for the first time that *S. japonicum* infection at delivery is also associated with elevated levels of endotoxin in maternal and placental plasma. These data suggest additional mechanisms by which schistosomiasis negatively impacts the maternal-fetal dyad.

1001

HELMINTH-INDUCED IL-4 ABOLISHES INKT CELL-MEDIATED CLEARANCE OF *BACTERIURIA*

Yi-Ju Hsieh, Chi-Ling Fu, **Michael Hsieh**

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

Infection with *Schistosoma haematobium*, the cause of urogenital schistosomiasis, is a risk factor for bacterial urinary tract co-infection. This co-infection worsens the sequelae of urogenital schistosomiasis, including hematuria, dysuria, and risk of bladder cancer. Despite the impact of these infections, it is unknown how co-infection by *S. haematobium* and bacterial uropathogens impairs host clearance of bacterial UTI. Many helminth infections, including schistosomiasis, induce host leukocytes to secrete IL-4. It is unknown whether IL-4 impairs bacterial clearance in schistosome-bacterial co-infections. Another ill-defined but key facet of anti-bacterial immunity is the role of iNKT cells. To study the mechanisms of *S. haematobium*-bacterial uropathogen co-infections, we combined the first tractable model of urogenital schistosomiasis with an established mouse model of bacterial UTI. This model recapitulates human co-infection, since a single bladder exposure to *S. haematobium* eggs triggers IL-4 production and renders a mouse strain susceptible to bacterial UTI when it otherwise is resistant (BALB/c). During co-infection, bladders are infiltrated by fewer iNKT cells than during bacterial UTI alone. Moreover, co-infection results in lower CD1d expression in bladder dendritic cells and lower levels of IFN-γ in bladder iNKT cells on a per-cell basis. We have found that three distinct conditions can restore the baseline resistance of BALB/c mice to bacterial UTI despite prior exposure to *S. haematobium* eggs: 1) antibody neutralization of IL-4, 2) genetic deficiency of IL-4 receptor-α signaling, and 3) exogenous gly*coli*pid antigen-induced activation of iNKT cells. We hypothesize that *S. haematobium* egg-induced IL-4 reduces CD1d expression by antigen-presenting cells, which dampens iNKT cell-derived, IFN-γ-mediated clearance of bacteriuria. Continuing work will test this hypothesis, and may enable iNKT cell-based therapies as alternatives to antibiotic treatment of UTI more broadly.

1002

SCHISTOSOMIASIS *JAPONICA* SOLUBLE EGG ANTIGENS INTERFERE WITH DIFFERENTIATION AND INVASION OF PLACENTAL TROPHOBLAST CELLS

Emily A. McDonald[1], Fusun Gundogan[2], Jennifer F. Friedman[1], Jonathan D. Kurtis[1]

[1]Rhode Island Hospital, Providence, RI, United States, [2]Women and Infants Hospital, Providence, RI, United States

Schistosomiasis represents a significant disease burden in endemic regions. We have previously shown that schistosomiasis during pregnancy results in a pro-inflammatory cytokine response detectable in maternal, placental, and cord blood as well as increased pathological signs of placental inflammation. Our previous data suggest this response is at least partly due to altered cytokine production by the trophoblast cells of the placenta. In addition to immune balance, trophoblasts are responsible for the majority of placental functions, including invasion of the uterine lining and hormone production for the maintenance of pregnancy. Herein, we have expanded our previous data to examine the effect of schistosome soluble egg antigens (SEA) on specific aspects of trophoblast function, including differentiation, invasion and hormone production. Primary cytotrophoblasts were collected at term from uninfected North American placentas and placed in culture for 5d, during which time they spontaneously differentiate to syncytiotrophoblast, the cell layer responsible for nutrient/gas/waste exchange and hormone production. Real-time qPCR for syncytin, a marker of trophoblast differentiation, showed a decrease in mRNA levels in cells exposed to SEA for the final

24h of the culture period. Progesterone production in this model system also tended to be lower in trophoblasts exposed to SEA. In addition, we used the HTR8/SVneo cell line as a model of extravillous trophoblast with invasive characteristics. Treatment of HTR8 cells with SEA for 24h resulted in a significant drop in the number of cells that invaded through a matrigel-coated membrane. Interestingly, there was no difference in either model system in the degree of TUNEL staining, suggesting SEA does not cause apoptosis in trophoblast cells. These data suggest that SEA interferes with the differentiation and function of trophoblast cells, and thus may predispose affected pregnancies to poor placentation and consequent birth outcomes.

1003

MATERNAL SCHISTOSOMIASIS *JAPONICA* INDUCES A FIBROGENIC RESPONSE IN NEONATES

Emily A. McDonald[1], Ling Cheng[1], Sunthorn Pond-Tor[1], Blanca Jarilla[2], Marianne Saglib[2], Analisa Gonzal[2], Remigio Olveda[2], Luz Acosta[2], Eric S. White[3], Jennifer F. Friedman[1], Jonathan D. Kurtis[1]

[1]Rhode Island Hospital, Providence, RI, United States, [2]Research Institute of Tropical Medicine, Manila, Philippines, [3]University of Michigan, Ann Arbor, MI, United States

The global burden of schistosomiasis is significant, with fibrosis one of the primary morbidities associated with the disease. Although the etiology and endpoints of fibrosis are thought to occur locally by surrounding eggs trapped in the liver, many molecules associated with fibrosis can be measured in the blood of infected individuals. We have previously shown that schistosomiasis during pregnancy results in a pro-inflammatory cytokine response in the cord blood of the affected neonate. In this study, we extended these findings to include a large panel of fibrogenic markers. A multiplex bead-based assay (FibroPlex v2) was developed in our laboratory to measure the levels of 35 mediators, effect modifiers, and outcomes associated with fibrosis. Cord blood from a cohort of 109 neonates born to mothers residing in an *Schistosoma japonicum* endemic area was assessed for all 35 of these fibrosis-related molecules. After adjusting for potential confounders, ten distinct pro-fibrotic mediators were significantly higher ($P < 0.05$) in the cord blood samples from infants whose mothers were infected with schistosomiasis during gestation (N=56) compared to infants born to uninfected mothers (N=53). These included IGF-1 (1.4 fold higher in neonates from infected mothers), TGF-β1 (2 fold higher), CTGF (2.7 fold higher), PICP (1.6 fold higher), ICTP (1.5 fold higher), collagen VI (1.3 fold higher), desmosine (1.8 fold higher), MMP-2 (4.4 fold higher), TIMP-1 (1.6-fold higher), and TIMP-4 (1.5 fold higher). Our data demonstrate that maternal schistosomiasis is sufficient to elicit a 'fibrogenic signature' in the cord blood of the neonate. As the first report of fibrosis-associated molecules altered in the newborn of infected mothers, these data have broad implications for the health of the developing fetus *in utero*, as well as after birth and into adulthood.

1004

DEEP PROFILING OF THE NOVEL INTERMEDIATE-SIZE NONCODING RNAS IN INTRAERYTHROCYTIC *PLASMODIUM FALCIPARUM*

Chunyan Wei[1], Tengfei Xiao[2], Zhensheng Wang[1], Peng Zhang[2], Xiaowei Chen[2], Runsheng Chen[2], Heng Wang[1]

[1]Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing, China, [2]Institute of Biophysics, Chinese Academy of Sciences, Beijing, China

Intermediate-size noncoding RNAs (is-ncRNAs) have been shown to play important regulatory roles in the development of several eukaryotic organisms. However, is-ncRNAs have not been thoroughly explored in *Plasmodium falciparum*, the most virulent malaria parasite that affects humans. In order to understand the whole profile of is-ncRNAs in *P. falciparum*, we performed a systematic identification of novel is-ncRNAs in intraerythrocytic *P. falciparum* 3D7 using Illumina/Solexa paired-end sequencing of an is-ncRNA-specific library. A total of 1,159 novel is-ncRNAs, including antisense, intergenic, and intronic is-ncRNAs were identified. Bioinformatics analyses indicated that the intergenic is-ncRNAs were the least conserved among eight different *Plasmodium* species, and antisense is-ncRNAs were more conserved than their sense counterparts. Thirty-six novel sno/scaRNAs were predicted, seven potential novel classes of is-ncRNAs were discovered by clustering analysis, and two novel internal motifs of intergenic is-ncRNAs were identified. The expression of selected novel is-ncRNAs was confirmed by RT-PCR and northern blotting assays. An obvious difference in the novel is-ncRNA expression profiles of the early and late intraerythrocytic developmental stages of the parasite was observed by Realtime PCR, suggesting the expression of the novel is-ncRNAs is regulated tightly in the parasite. Many of the novel is-ncRNAs showed a higher expression in the early stage than in the late stage, implying their role in building the components and structures used in later stage. The expression levels of four antisense RNAs in our study were shown to be co-regulated with that of their *cis*-encoded sense RNAs, suggesting that these antisense RNAs are involved in the regulation of gene expression in the parasite. This study provides indispensible information to the whole noncoding transcriptome of the parasite and will help further function study of the novel is-ncRNAs in during the intraerythrocytic development of *P. falciparum*.

1005

ON THE ORIGIN OF ANTIGEN SEQUENCE DIVERSITY IN *PLASMODIUM FALCIPARUM*: VAR GENES UNDERGO FREQUENT MITOTIC RECOMBINATION

William L. Hamilton, Antoine Claessens, Mihir Kekre, Adnan Faizullabhoy, Julian Rayner, Dominic Kwiatkowski

Wellcome Trust Sanger Institute, Hinxton, United Kingdom

Plasmodium falciparum is a unicellular parasite responsible for severe human malaria. The parasite divides asexually within infected red blood cells (iRBCs), and coats the erythrocyte surface with highly polymorphic proteins in the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family. These are encoded by ~60 var genes per genome, and play an important role in immune evasion through antigenic variation and parasite sequestration to the microvasculature. We investigated how diversity is generated in var genes using a clonal dilution system to isolate single iRBCs, followed by whole genome next generation DNA sequencing of the expanded populations derived from these single cells. We cultured three *P. falciparum* strains from diverse geographic origins over 4-8 months, sub-cloning every 4-8 weeks, and sequenced 106 samples at high coverage. Single Nucleotide Polymorphisms (SNPs) were distributed throughout the genome at a rate of ~2x10^-9 SNP/generation/nucleotide, suggesting that a point mutational process does not contribute to var gene diversity. In contrast, all of the 41 structural variants identified in coding regions occurred within var genes, and produced chimeric sequences through gene conversions and duplications. Recombination occurred within 'identity blocks' (IBs) - runs of 4-48bp (average: 15bp) of identical sequences between the recombining var genes, though no conserved motif was observed. The ~80bp surrounding the IB mid-point were more homologous than the background var gene level, and the resulting chimeric var genes were kept in frame with no SNPs or InDels. These data suggest that homology-dependent DNA repair is responsible for var gene recombination in mitosis, and we speculate that this process has evolved to increase var gene sequence diversity and produce novel PfEMP1 antigens. This has direct implications for the parasite's survival in semi-immune malaria patients.

1006

EXPRESSION VARIATION IN *PLASMODIUM FALCIPARUM*: IMPLICATIONS FOR PARASITE ADAPTATION

Lindsey B. Turnbull, Geoffrey Siwo, Asako Tan, Michael T. Ferdig

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

Phenotypic plasticity is crucial in biological systems because it allows populations to adapt to constantly changing environments. Phenotypic heterogeneity within populations of genetically identical cells is an important but often overlooked source of phenotypic plasticity. Most investigations currently focus on the average of quantitative traits; however, several recent studies in model organisms have demonstrated that variation in expression is an adaptive genetically controlled phenotype that can be repeatedly measured. Thus, we anticipate expression variation will be important for adaptation in *Plasmodium falciparum*. Even small variations in transcriptional abundance among the millions of parasitized red blood cells within a single malaria infection may provide a selective advantage to a subset of the parasite population, thereby enhancing parasite fitness. To study the innate biological expression variation in the malaria parasite, we compared the gene expression variance within five sub-cloned lines of HB3, Dd2, and select progeny from the cross. For each parasite sub-clone, genome-wide gene expression levels were obtained using a custom designed high density exon array. We observed that parasite sub-clones from the same line have diverging gene expression profiles when measured at the same developmental stage and environmental conditions. For example, gene expression variation within HB3 sub-clones was much higher than within Dd2 sub-clones. This could be related to the strong selection bottlenecks that Dd2 has undergone, including drug selection pressure under mefloquine and chloroquine. It could also indicate that drug resistant parasites are more susceptible to rapidly changing environments. The distinct gene expression variation within the sub-clones of the parental lines opens the possibility that their progeny will show varying levels of transcriptional plasticity. Therefore, our extended study could lead to the discovery of genetic determinants of this plasticity, a potentially important factor in the adaptability of the parasite to drugs.

1007

FUNCTIONAL ANALYSES OF SPOROZOITE RHOPTRY PROTEINS BY STAGE-SPECIFIC GENE SILENCING SYSTEM IN *PLASMODIUM BERGHEI*

Tomoko Ishino[1], Yuka Sugino[1], Naohito Tokunaga[2], Mamoru Nozaki[1], Mayumi Tachibana[1], Takafumi Tsuboi[3], Motomi Torii[1]

[1]Division of Molecular Parasitology, Proteo-Science Center, Ehime University, Toon, Ehime, Japan, [2]INCS, Ehime University, Toon, Ehime, Japan, [3]Division of Malaria Research, Proteo-Science Center, Ehime University, Matsuyama, Ehime, Japan

Malaria transmission to mammalians is initiated by sporozoites inoculation into the skin via mosquito bite. Sporozoites migrate to the liver through the blood vessel then invade hepatocytes within parasitophorous vacuole, where they develop into thousands of erythrocyte invasive forms. Rhoptry is one of the apical organelles observed only in sporozoites and merozoites. Recently, it has been suggested that rhoptry proteins are localized to the tight junction and have important roles during merozoite invasion of erythrocyte. Here, we focused on sporozoite rhoptry proteins to elucidate the molecular mechanisms of sporozoite infectious ability. Since it was shown that 8 proteins are expressed and localized to rhoptries both in merozoites and sporozoites, we intend to analyze their functions during sporozoite infection of target cells by reverse genetics. Therefore, we generated stage specific gene silencing transgenic parasites for each gene by replacing the endogenous promoter to the merozoite-specific promoter. In vivo and *in vitro* infectivity analyses of mutant sporozoites reveal that some proteins are required for salivary gland invasion and others for liver invasion. This is the first reverse genetic evidence to show that rhoptry proteins are involved in target cell infection.

1008

ESSENTIAL ROLE FOR A KINASE IN *PLASMODIUM FALCIPARUM* GAMETOCYTE PRODUCTION

Belinda J. Morahan[1], Jean Halbert[2], Omar Ali[3], Beata Czesny[3], Jose Garcia-Bustos[1], Christian Doerig[1], Kim C. Williamson[3]

[1]Monash University, Clayton, Australia, [2]Global Health Institute, Lausanne, Switzerland, [3]Loyola University Chicago, Chicago, IL, United States

Malaria transmission from an infected human host to a mosquito vector relies on the production of male and female gametocytes. Despite the crucial role that these sexual stage parasites have in the malaria lifecycle, little is known about the triggers or molecular mechanisms that mediate sexual development. We have previously shown that *Plasmodium falciparum* gametocyte development 1 gene (*Pfgdv1*) is critical for early sexual differentiation; however other genes are likely to also be required for gametocyte production. Candidates include kinases that could be involved in the initiation or propagation of the cascade of gene activation that is associated with sexual development. Here we report on the localisation of PFB0665w, a predicted serine/threonine kinase that has a transcription profile similar to *Pfgdv1,* in sexually committed rings and stage I-IV gametocytes, using immunofluorescence microscopy of transgenic parasites expressing the HA-tagged enzyme. Targeted disruption of *PFB0665w* eliminated gametocyte production, suggesting the gene is critical for sexual differentiation. This is consistent with our previous results showing that *PFB0665w* is likely dispensable for asexual blood stage *P. falciparum* infection. Reverse-transcription quantitative PCR confirmed expression of *Pfgdv1* in the *PFB0665w* minus cell line, indicating that it is not required for *Pfdgv1* expression. Based on our data, we suggest PFB0665w is essential for gametocyte production and that it functions downstream of Pfgdv1 in the gametocytogenesis cycle. This role together with its continuous expression throughout sexual differentiation potentially makes PFB0665w an attractive target for a transmission-blocking strategy to eliminate malaria. We are currently investigating if the kinase is also expressed in schizonts whose progeny is pre-committed to gametocytogenesis, as is the case for the NIMA-related kinase Pfnek-4.

1009

THE ROLE OF GLUTATHIONE BIOSYNTHESIS IN MODULATING DRUG RESPONSE IN *PLASMODIUM FALCIPARUM*

Angana Mukherjee, Daria Van Tyne, Dyann Wirth, Sarah K. Volkman

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

Glutathione, a tripeptide (γ-glutamylcysteinyl-glycine, GSH) is a major reduced thiol in *Plasmodium* with important functions as a redox buffer and as a cofactor for detoxifying enzymes. GSH is synthesized in *Plasmodium* in two steps: the first and the rate-limiting step is catalyzed by gamma-glutamyl cysteine synthetase (γ-GCS) followed by glutathione synthetase (GS). The genes encoding these proteins are believed to be essential in *Plasmodium falciparum*. Modulation of γ-GCS and the antioxidant network have been recently reported to be associated with artemisinin and dihydroartemisinin resistance in *P. yoelii* and *P. falciparum* and interestingly; in a recent genome-wide association study (GWAS) we observed associations between the non-synonymous single nucleotide polymorphisms (SNPs) K173I in γ-GCS and I376L in GS and parasite response to dihydroartemisinin. We also sequenced the gene encoding γ-GCS and observed a variable repetitive motif of (Y/C)QS(N/D)LQQQ, repeating in tandem between 5X-15X in 60 strains of *P. falciparum* worldwide. This length polymorphism was not associated to *in vitro* culture adaptation, since the repetitive motifs were identical in paired *ex vivo* and culture adapted parasites. While in 22% of these strains, including 3D7, the sequence motif was present only once, we observed a significant association between length polymorphism of γ-GCS and parasite response to lumefantrine, halofantrine, and mefloquine in 25 Senegalese clinical isolates, suggesting that length polymorphism in this gene may have a functional role. To understand the importance of the different variants

of γ-GCS and GS, we overexpressed HA-tagged versions of the wild-type and mutant γ-GCS and GS alleles in 3D7 parasites. Western blot analysis confirmed the expression of HA-tagged γ-GCS and GS across the life cycle, with protein expression peaking at early trophozoite stage. We observed that overexpression of γ-GCS conferred resistance to L-buthionine sulfoximine (BSO), an irreversible inhibitor of γ-GCS. We are currently studying the levels of GSH, accumulation of reactive oxygen species (ROS) and the responses of these over-expressors to several antimalarials and to agents that induce oxidative stress and testing the hypothesis if specific antimalarial agents work by modulating the GSH bio-synthetic pathway.

1010

PROTEIN HOMEOSTASIS AS A NOVEL TARGET FOR ANTIMALARIAL THERAPY: A STUDY OF THE PROLYL-TRNA SYNTHETASE INHIBITOR HALOFUGINONE IN *PLASMODIUM FALCIPARUM*

Jonathan D. Herman[1], Lauren Pepper[2], Joseph F. Cortese[1], Kevin Gallinsky[1], Tracey Keller[3], Malcolm Whitman[3], Susan Lindquist[2], Ralph Mazitschek[4], Dyann F. Wirth[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Whitehead Institute for Biomedical Research, Cambridge, MA, United States, [3]Harvard School of Dental Medicine, Boston, MA, United States, [4]Massachusetts General Hospital, Boston, MA, United States

Many current anti-malarial drugs work within the same biological pathways leading to shared resistance mechanism. We have taken the methodology of chemogenomics to identify potential antimalarials that target novel pathways. Understanding the anti-plasmodial mechanism of halofuginone (HFG), a febrifuginone analogue, informs our understanding of parasite biology and directs the future creation of novel therapies. To interrogate mechanism, we selected parasites that are resistant to halofuginone and then used whole genome sequencing to identify the causative mutation (SNPs) and developed high resolution melting (HRM) genotyping assays to follow up those most promising. We found two nonsynonomous mutations in the active site of the cytoplasmic prolyl-tRNA synthetase (*Pf*cPRS) in independent selections and have validated PfcPRS as the target of Halofuginone using a heterologous *S. cerevisiae* model, recombinantly expressed protein, and *Plasmodium falciparum* reverse genetics. Now, we have further characterized the role of the halofuginone and related stresses on amino acid starvation and other mechanisms of protein homeostasis in *P. falciparum*. Treatment of parasites with halofuginone and febrifugine results in increased phosphorylation of a *P. falciparum* eIF2α analogue. Overall, these results demonstrate halofuginone-induced proline starvation via an interaction with *Pf*cPRS leads to translational inhibition. Thus we posit that amino acid supply and aminoacyl tRNA synthetases are a new and promising target for chemotherapeutic intervention.

1011

FUNCTIONAL ROLES FOR C5A AND C5AR, BUT NOT C5L2, IN THE PATHOGENESIS OF HUMAN AND EXPERIMENTAL CEREBRAL MALARIA

Hani Kim[1], Laura K. Erdman[1], Ziyue Lu[1], Lena Serghides[1], Kathleen Zhong[1], Aggrey Dhabangi[2], Charles Musoke[2], Craig Gerard[3], Christine Cserti-Gazdewich[4], W. Conrad Liles[5], Kevin C. Kain[1]

[1]University of Toronto, Toronto, ON, Canada, [2]Makerere University, Kampala, Uganda, [3]Harvard Medical School, Boston, MA, United States, [4]Toronto General Hospital - University Health Network, Toronto, ON, Canada, [5]University of Washington, Seattle, WA, United States

The host immune response plays an important role in the onset and progression of cerebral malaria (CM). The complement system is an essential component of the innate immune response to malaria, and its activation generates the anaphylatoxin, C5a. To test the hypothesis that C5a signaling contributes to the pathogenesis of CM, we examined plasma levels of C5a in children with CM vs. uncomplicated malaria (UM), and investigated a causal role for the C5a receptors, C5aR and C5L2, in a mouse model of experimental CM (ECM) induced by *Plasmodium berghei* ANKA (PbA) infection. In a nested case-control study of Ugandan children, the median levels of C5a at presentation were significantly higher in children with CM versus those with UM (43.7 vs. 22.4 ng/mL; $p < 0.001$). In the ECM model, *C5aR-/-* mice displayed significantly improved survival compared to their wild-type (WT) counterparts ($p=0.004$); whereas *C5L2-/-* mice showed no difference in survival from WT mice. Improved survival in *C5aR-/-* mice was associated with reduced levels of pro-inflammatory cytokines TNF, IFN and MCP-1. Furthermore, endothelial quiescence and integrity in the brain were enhanced as demonstrated by increased levels of angiopoietin-1, decreased levels of angiopoietin-2 and soluble ICAM-1, and decreased Evans blue extravasation. These findings demonstrate that C5a is dysregulated in human CM and contributes to the pathogenesis of ECM via C5aR-dependent inflammation and endothelial dysfunction.

1012

RAPID, PITTING-INDEPENDENT CLEARANCE OF *PLASMODIUM FALCIPARUM* IN IMMUNE MALIAN CHILDREN TREATED WITH ARTESUNATE FOR UNCOMPLICATED MALARIA

Papa Alioune Ndour[1], Tatiana Lopera-Mesa[2], Stéphane Jauréguiberry[3], Seidina Diakité[1], Serena Chang[2], Eric Kendjo[4], Oussama Mouri[1], Sylvestre Biligui[1], Antoine Claessens[5], Camille Roussel[1], Liliane Ciceron[1], Dominique Mazier[6], Marc Thellier[7], Mamadou Diakité[8], Rick Fairhurst[2], Pierre Antoine Buffet[9]

[1]Inserm-UPMC (Paris 6 University) UMRs945, Paris, France, [2]Laboratory of Malaria and Vector Research, National Institutes of Health, Rockville, MD, United States, [3]Infectious Disease Unit, Inserm-UPMC (Paris 6 University) UMRs945, Pitié Salpêtrière Hospital APHP, Paris, France, [4]CNR Paludisme, Paris, France, [5]University of Edinburgh, Edinburgh, United Kingdom, [6]Inserm-UPMC (Paris 6 University) UMRs945, Mycology Parasitology Unit, Paris, France, [7]CNR Paludisme, Mycology Parasitology Unit, Hôpital Pitié-Salpêtrière, APHP, Paris, France, [8]University of Bamako, Bamako, Mali, [9]Inserm-UPMC (Paris 6 University) UMRs945, Mycology Parasitology Unit, APHP, Paris, France

In patients with *Plasmodium falciparum* malaria, artemisinin-induced clearance of infected red blood cells (iRBCs) is faster than clearance induced by other antimalarial drugs. This difference has been attributed to "pitting," a spleen-specific process whereby parasites altered by artemisinins are expelled from their host RBCs. After pitting, once-infected RBCs (O-iRBCs) are released intact into the circulation. Parasite clearance time (PCT) and peak levels of O-iRBCs in the circulation were analyzed in French travelers with severe malaria, and in artemisinin-treated Malian children with uncomplicated malaria. O-iRBCs peaked at 95% and 25% of initial *parasitemia* in artesunate- and quinine-treated travelers, thus confirming the major role of pitting in artemisinin-induced parasite clearance in non-immune patients. In Malian children, mean PCT was short (20 hours) and significantly shorter in 9- to 13-year-old children than in 0.5- to 4-year-old children (14 vs. 26 hours, $p=0.0001$). As expected, peak O-iRBC levels were significantly lower in older than in younger children (27.2% vs. 93.9%) suggesting that the very short PCT in older children was in part unrelated to pitting rate. In Malian children, the proportion of iRBCs recognized by autologous IgG ex vivo correlated significantly with PCT ($r=-0.501$, $p=0.0006$) and peak O-iRBC levels ($r=-0.420$, $p=0.0033$). Lag phases on parasite clearance curves were shorter in older children and infants, suggesting the existence of an immune-dependent clearance mechanism occurring faster than pitting in infants with passively-acquired humoral immunity from their mother and in older children with multiple previous exposures to *P. falciparum*. Mechanisms of *P. falciparum* clearance in artemisinin-treated patients may be more diverse than previously thought.

1013

MALARIA INFECTION DEPLETES HEPATIC DDAH1, A REGULATOR OF ENDOTHELIAL NITRIC OXIDE SYNTHESIS

Jessica H. Chertow, Matthew Alkaitis, Glenn Nardone, Hans Ackerman

National Institutes of Health, Rockville, MD, United States

Impaired endothelial nitric oxide (NO) synthesis is associated with the clinical severity and risk of death from malaria. Endothelial NO synthesis is dependent upon the blood levels of arginine, the substrate for NO synthesis, and asymmetric dimethylarginine (ADMA), a potent inhibitor of NO synthesis. Homeostasis of the arginine to ADMA ratio is maintained by hepatic dimethylarginine dimethylaminohydrolase-1 (DDAH1), an enzyme that metabolizes ADMA at a rate inversely proportional to arginine concentration. A genome-wide association study recently identified a polymorphism in DDAH1 to be associated with susceptibility to severe malaria in Gambian children. We hypothesized that malaria infection causes DDAH1 dysfunction, dysregulation of the arginine to ADMA ratio, and impaired NO synthesis. To test this hypothesis, we infected C57Bl/6 mice with *Plasmodium berghei* ANKA, an established model of severe malaria. DDAH1 protein concentration was determined by quantitative analysis of Western blots of liver homogenates. Tissue and plasma concentrations of ADMA and arginine were determined by HPLC. Nitrite, an indicator of NO synthesis, was measured by gas-phase chemiluminescent assay. Data represent 3 independent experiments. Six days after inoculation with *P. berghei*, protein levels of DDAH1 fell by 55% compared to uninfected controls ($p < 0.0001$). Concurrently, the proportion of arginine to ADMA fell from 118 ± 28 in control animals to 87 ± 22 in infected animals ($p < 0.001$). Blood nitrite levels fell from 0.53 ± 0.1 uM in control animals to 0.36 ± 0.1 uM in infected animals, consistent with impaired NO synthesis ($p < 0.01$). This experimental model of severe malaria recapitulated the decreased arginine to ADMA ratio and decreased nitrite levels observed in West African children with severe malaria. Analysis of hepatic tissue revealed loss of DDAH1 protein to be a potential mechanism explaining the dysregulation of ADMA metabolism and the impairment of NO synthesis that occurs during severe malaria. These findings encourage further investigation into the naturally occurring human genetic variants of DDAH1 that determine susceptibility to severe malaria in African children.

1014

ANTI-SELF ANTIBODIES AGAINST PHOSPHATIDYLSERINE INDUCE ANEMIA IN MALARIA

Cristina Fernandez-Arias, Ana Rodriguez

New York University Langone Medical Center, New York, NY, United States

Severe malarial anemia is one of the leading causes of mortality in malaria. *Plasmodium* invades red blood cells (RBCs) to mature and reproduce, but this represents only a minimal loss compared to the massive elimination of uninfected RBCs, which contributes decisively to anemia in malaria . Using a mouse model, we have found that *Plasmodium* infection induces the generation of $CD4^+$ T cell-dependent anti-self antibodies that bind to the surface of infected and uninfected RBCs from infected animals, but not from control uninfected mice. Phosphatidylserine (PS), which is exposed on the surface of a high fraction of uninfected RBCs during malaria, is recognized by these antibodies, facilitating their phagocytosis by macrophages. When anti-self anti-PS antibodies are transferred into *Plasmodium* infected mice, a significant increase in anemia is observed, which is not found when they are transferred into control uninfected animals or when irrelevant antibodies are transferred. Conversely, blocking of PS in infected mice through the injection of annexin V results in a faster recovery from anemia. These findings indicate that autoimmune antibodies induced by malaria, recognize PS on the surface of uninfected erythrocytes and mediate their clearance, contributing to anemia. Inhibition of this pathway may be potentially exploited for treating malarial anemia.

1015

INVESTIGATING THE ANGIOPOIETIN-TIE2 PATHWAY AS A THERAPEUTIC TARGET TO IMPROVE SURVIVAL FOLLOWING EXPERIMENTAL LIFE-THREATENING *PLASMODIUM* CHALLENGE

Sarah J. Higgins[1], Karlee L. Silver[2], W. Conrad Liles[3], Kevin C. Kain[4]

[1]Department of Laboratory Medicine and Pathobiology, University of Toronto/Sandra Rotman Centre for Global Health, University Health Network, Toronto, ON, Canada, [2]Sandra Rotman Centre for Global Health, University Health Network, Toronto, ON, Canada, [3]University of Washington, Seattle, WA, United States, [4]Sandra Rotman Centre for Global Health, University Health Network; University of Toronto, Toronto, ON, Canada

Cerebral malaria (CM) pathogenesis is associated with endothelial activation and loss of blood brain barrier integrity. The angiopoietin (Ang)-Tie2 signalling pathway is a key regulator of endothelial function. Alterations in the angiogenic balance, specifically increased Ang-2 relative to Ang-1, has been associated with poor clinical outcome in CM. It remains unclear whether the Ang/Tie2 pathway is causally involved in CM pathogenesis. We hypothesize that dysregulation in angiopoietins contributes to ECM pathogenesis, and interventions to maintain Tie2 activation may promote endothelial stability, prevent deleterious alterations to the BBB and improve outcome following *Plasmodium* infection. Using the murine model of *Plasmodium berghei* ANKA (PbA)-induced experimental CM (ECM), we show that altered protein and mRNA levels of angiopoietins are associated with disease severity, similar to observations in human populations, and directly precede the loss of vascular integrity in the brain and the onset of severe neurological symptoms, such as seizures and paralysis. Therapeutic intervention to maintain Tie2 activation (e.g. Adenoviral expression of Ang-1) significantly improved survival in ECM-susceptible C57Bl/6 mice compared to empty adenoviral vector controls and vehicle controls ($p=0.001$, logrank test) and prevented ECM-induced neurological impairment. The survival benefit was further increased when therapeutic Ang1 was delivered in combination with a sub-curative dose of the anti-parasitic drug artesunate ($p=0.0039$) compared to the benefit of artesunate alone. Conversely, genetic deletion using a conditional Cre/loxP system showed that moderately resistant Balb/c mice have significantly worsened disease outcome in the absence of normal Ang/Tie2 interaction ($p=0.01$), further supporting a critical role of angiogenic-Tie2 signaling during ECM. Overall, these findings underscore the contribution of the angiopoietin-Tie2 pathway to the pathobiology of ECM and show that adjunctive treatment strategies based on promoting endothelial quiescence improve survival in ECM.

1016

CHARACTERIZATION OF THE DIFFERENTIAL INVASION OF *PLASMODIUM FALCIPARUM* INTO YOUNG AND OLD RBCS

Martha A. Clark[1], Morgan M. Goheen[1], Raj S. Kasthuri[1], Timothy J. McMahon[2], Carla Cerami[1]

[1]University of North Carolina Chapel Hill, Chapel Hill, NC, United States, [2]Duke University, Durham, NC, United States

The blood stage of a malaria infection is responsible for all disease, and *Plasmodium falciparum* is the most pathogenic of all the malaria species to infect humans. *P. falciparum* is capable of infecting all host RBCs regardless of age, however infection rate of young RBCs is greater than old RBCs. RBC physiology changes during the course of the 120 day RBC lifespan, culminating in the signals required to trigger clearance of senescent RBCs from circulation. As RBCs age cell volume, enzymatic activity, deformability, membrane protein abundance and sialation decrease while cell density, osmotic fragility, and oxidative damage increase. We show that the abundance of important RBC *P. falciparum* invasion ligands Basinigin (CD147) and CR-1 (CD35) decrease with increasing RBC age. Furthermore, we utilize a novel three color invasion assay to investigate the

contribution of (i) RBC *P. falciparum* invasion ligands (ii) sialation of RBC surface proteins, and (iii) membrane rigidity on the differential invasion of *P. falciparum* into young and old RBCs. We show that decreased density of RBC invasion ligands on old RBCs does not contribute to the decreased invasion rate of *P. falciparum* into old RBCs, but that differences in RBC membrane rigidity and membrane protein sialation do influence the differential invasion of young and old RBCs by *P. falciparum.* Our investigation emphasizes the dynamic nature of the host RBC population and the contribution that changing RBC physiology has on *P. falciparum* infection.

1017

USE OF A NOVEL FLUORESCENT-LABELED SINGLE CHAIN ANTIBODY FOR PARATRANSGENIC CONTROL OF LEISHMANIASIS

Ivy Hurwitz[1], Annabeth Fieck[1], Angray Kang[2], Marcelo Ramalho-Ortigao[3], Ravi Durvasula[1]

[1]University of New Mexico and New Mexico Veterans Affairs Health Care System, Albuquerque, NM, United States, [2]Queen Mary University of London, Barts and The London School of Medicine and Dentistry, London, United Kingdom, [3]Kansas State University, Manhattan, KS, United States

Leishmaniasis is caused by protozoan parasites of the genus *Leishmania*, and is transmitted by the bite of an infected Phlebotomine sand fly. This disease is a leading cause of morbidity in the world, with close to 12 million people infected in over 80 countries worldwide. Transmission control is heavily dependent on the use of chemical pesticides. However, environmental toxicity, adverse effects on human health and the emergence of insect resistance have greatly undermined their efficacy. We have reported on a paratransgenic strategy to control vectorial transmission of this neglected tropical disease in previous work. Here we describe the development of a novel effector molecule for use in this paratransgenic approach. The monoclonal antibody B72.3 binds to sialyl-(le)a glycan, and was demonstrated to bind with specificity to the surface of *L. donovani, L. mexicana* and *L. major* promastigotes. We have generated a recombinant single chain antibody (scFv) of B72.3, and have demonstrated that it binds with specificity to several *Leishmania spp.* We have subsequently replaced the linker region between the VH and VL domains with monomeric red fluorescent protein mRFP from Discosoma, generating a fluorescent REDantibody. There are numerous reports citing the release of reactive oxygen species (ROS) following illumination of photosensitizer proteins such as mRFP. By linking the mRFP to the B72.3 scFv, we have targeted this ROS release to invading *Leishmania* promastigotes. We hypothesize that exposure of the paratransgenic sand fly to the intense light conditions in their native environments would lead to the release of ROS by the B72.3 REDantibody. These molecules are expected to result in membrane damage, leading to decrease parasite viability in the paratransgenic sand fly. We have validated that this modified version of the B72.3 scFv binds with specificity to three *Leishmania spp.* In preliminary studies, we were able to detect ROS release from the B72.3 REDantibody following exposure to a halogen light source. The ability of the ROS release following light exposure to decrease parasite load *in vitro* are currently underway utilizing mid-gut binding assays.

1018

LEISHMANIA INFLUENCE DIFFERENTIAL MICRORNA EXPRESSION PROFILES WITH IMMUNOLOGICALLY CRITICAL TARGET TRANSCRIPT NETWORKS AMONG HOST CELLS

Nicholas S. Geraci, Rory D. Carmichael, John C. Tan, Mary A. McDowell

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

Leishmania donovani (*Ld*) and *L. major* (*Lm*) are vector-borne intracellular protozoan parasites and two Old World causative agents of visceral and cutaneous leishmaniasis respectively. These pathogens avoid innate immune destruction when parasitizing host macrophages (MP) or dendritic cells (DC), in part, by eliciting unique host cell type-specific transcriptional profiles. We explored the role of translational regulation by host microRNAs (miRNAs) in a *Leishmania* species specific manner via RNAseq, microarray, and quantitative proteomics analyses. Total mature miRNA expression profiles were explored through next generation sequencing of small RNAs (<30nt) isolated from a time course of DC and MP *in situ* infections with *Lm* and *Ld* separately. Expression profiles of large coding RNAs was assessed using microarray; and protein expression was determined via quantitative mass spectrometry of matched donor samples. Results of integrative multi-dimensional dataset analyses revealed differential miRNA expression profiles which were host cell type and infecting parasite species specific. Most miRNAs were downregulated across infection conditions compared to uninfected controls. Among DC infected with *Ld*, a small selective miRNA group was upregulated. Correlative target prediction analysis identified negative TGFβ pathway regulator transcripts as the primary targets of *Ld* upregulated miRNAs. miRNA promoter analysis also revealed docking sites for TGFβ induced transcription factors. Functional studies are underway to confirm these target predictions and assess the potential for TGFβ pathway dysregulation by *Ld* induced miRNAs as a permissive mechanism for *Leishmania* parasite survival. This study serves as a pilot for further investigations into the differentially selective functions of host cell miRNAs during *Ld* and *Lm* infection.

1019

CHARACTERIZATION OF CROSS PROTECTION BY GENETICALLY MODIFIED LIVE ATTENUATED *LEISHMANIA DONOVANI* PARASITES AGAINST CUTANEOUS LEISHMANIASIS CAUSED BY *L. MEXICANA*

Ranadhir Dey[1], Gayathri Natarajan[2], Hannah Cummings[2], Angamuthu Selvapandiyan[3], Robert Duncan[1], Abhay R. Satoskar[2], Hira L. Nakhasi[1]

[1]CBER/Food and Drug Administration, Bethesda, MD, United States, [2]Wexner Medical Center at the Ohio State University, Columbus, OH, United States, [3]Institute of Molecular Medicine, Columbus, OH, United States

Leishmaniasis causes significant morbidity and mortality worldwide and there is no vaccine against this disease. Previously we showed that genetically modified live attenuated *L. donovani* parasite cell lines ($Cen^{-/-}$ and $p27^{-/-}$) induce a strong cellular immune response providing protection against visceral leishmaniasis in mice. In the current study, we show that both these cell lines induce a strong pro-inflammatory response and stimulate the nitric oxide production in bone marrow derived mouse dendritic cells in contrast to wild type parasites. Upon challenge with wild type *L. mexicana*, mice immunized either for short (6 weeks) or long (8 months) periods showed significantly smaller lesions and lower parasite burden than naïve mice. Immunized and challenged mice showed a well organized collagen deposition indicating wound healing, whereas naïve mice had a dispersed and sparse collagen bundle after challenge indicated a growing lesion. Immuno-histochemical analysis of mice ear lesions from immunized and challenged mice showed significant influx of macrophages, MHCII expressing cells and nitric oxide producing cells. Further, after virulent challenge, the presence of de-granulated mast cells in lesions of non-immune mice, but not in immunized mice, confirmed parasite control by immunization. Cytokine analysis of the *L. donovani* antigen stimulated splenocyte culture supernatants from live attenuated parasite immunized, *L. mexicana* challenged mice revealed induction of both secreted IFNγ and IL4, suggesting a systemic mixed Th1 and Th2 immune response against the immunizing agent. However, *L. mexicana* antigen stimulated lymph node cell culture supernatants from immunized challenged mice revealed higher IFNγ secretion and suppression of IL10, IL4, IL5, IL13 and IL6 compared to non-immunized challenged mice suggesting attenuated *L. donovani* can provide protection against *L. mexicana* parasites by induction of a strong Th1 and suppressed Th2 cytokine response. These studies demonstrate the potential of live attenuated *L. donovani* parasites as pan *Leishmania* vaccines.

1020

CANDIDATE BIOMARKERS PREDICT PROGRESSION TO CHAGAS HEART DISEASE IN RURAL SOUTHERN BOLIVIA

Eva H. Clark[1], Morgan Marks[2], Robert H. Gilman[3], Antonio Fernandez[4], Thomas C. Crawford[5], Aaron M. Samuels[6], Gerson Galdos-Cardenas[7], Gilbert S. Menacho[8], Ricardo W. Bozo-Gutierrez[9], Diana L. Martin[6], Caryn Bern[10]

[1]University of Alabama at Birmingham, Birmingham, AL, United States, [2]Department of Epidemiology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, United States, [3]Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [4]Department of Cardiology, Rhode Island Hospital, Brown University School of Medicine, Providence, RI, United States, [5]Department of Cardiology, University of Michigan Health System, Ann Arbor, MI, United States, [6]Centers for Disease Control and Prevention, Atlanta, GA, United States, [7]Hospital Universitario Japones, Santa Cruz, Plurinational State of Bolivia, [8]Centro de Salud Eiti, Gutierrez, Plurinational State of Bolivia, [9]Hospital Municipal Camiri, Camiri, Plurinational State of Bolivia, [10]Global Health Sciences, University of California San Francisco, San Francisco, CA, United States

Nearly 30% of *Trypanosoma cruzi*-infected individuals eventually manifest Chagas cardiomyopathy (CC). A test for early detection of patients who will progress to CC is needed, as early treatment may improve prognosis. In this cross sectional study of a cohort in southern Bolivia, serum biomarkers and EKG changes were measured in 68 *T. cruzi*-positive individuals representing the various stages of *T. cruzi*-related heart disease and 17 individuals who were *T. cruzi*-negative and without evidence of cardiac disease. Chagas disease staging was assessed using the ACC/AHA heart failure classification system. Patients were classified as Stage A (*T. cruzi* positive with normal EKG/CXR), Stage B (*T. cruzi* positive with abnormal EKG but normal CXR), and Stage C (*T. cruzi* positive with abnormal EKG and cardiac structural changes). Markers Transforming Growth Factor (TGF) β1 and 2, Connective Tissue Growth Factor (CTGF), Matrix Metalloproteinase (MMP)2, MMP9, Tissue Inhibitor of Metalloproteinase (TIMP) 1, Brain Natriuretic Peptide (BNP), Mannose Binding Lectin (MBL), C-terminal Propeptide of Type I Procollagen (PICP), and N-terminal Procollagen III Propeptide (PIIINP) were measured using ELISA or Luminex. QRS scar score was calculated for each patient using EKG measurements. Individuals with Stage B had higher QRS Score ($p<0.001$), increased concentrations of MMP2 ($p=0.013$), TIMP1 ($p=0.003$), TGFβ1 ($p=0.023$), and TGFβ2 ($p<0.001$) as compared to those with Stage A. Multinomial logistic regression showed a strong association of increased concentrations of TIMP1 (RRR=1.94; $p=0.036$), TGFβ1 (RRR=2.05; $p=0.011$), and TGFβ2 (RRR=8.11; $p=0.037$) with a diagnosis of Stage B as compared to Stage A. A higher QRS Score (RRR=10.5; $p<0.001$) and increased concentrations of MMP2 (RRR=6.77; $p<0.001$) and BNP (RRR=2.75; $p=0.010$) were strongly associated with a Stage C diagnosis as compared to Stage A. Associations with markers of fibrosis and tissue remodeling (QRS Score, MMP2, and TIMP1) likely indicate the presence of advancing cardiac structural change. However, the elevated concentrations of immunoregulatory markers (TGFβ1 and TGFβ2) among those with Stage B disease may be clinically useful predictors of eventual development of CC. This information will be particularly important in the developing countries where Chagas disease is most prevalent and medical resources are limited.

1021

HIGH-THROUGHPUT SCREEN OF THE *TRYPANOSOMA BRUCEI* METHIONYL-TRNA SYNTHETASE ENZYME: INITIAL AND FOLLOW UP RESULTS

Frederick S. Buckner[1], Laura Pedro-Rosa[2], Ranae M. Ranade[1], John R. Gillespie[1], Christophe C. Verlinde[1], Erkang Fan[1], Wim G. Hol[1], Christina Eberhart[2], Thomas Bannister[2], Peter Hodder[2]

[1]University of Washington, Seattle, WA, United States, [2]The Scripps Research Institute, Jupiter, FL, United States

Improved treatments for human African trypanosomiasis are urgently needed to replace existing drugs that have major liabilities including poor efficacy, high toxicity, parenteral administration, or high costs. In previous work, we have demonstrated by RNA interference that methionyl-tRNA synthetase (MetRS) is essential for growth of the etiologic agent, *Trypanosoma brucei.* In order to screen for new chemical compounds with inhibitory activity against the *T. brucei* MetRS we developed a high-throughput enzyme assay based on ATP-depletion and a bioluminescence readout. After miniaturization of the assay to the 1536-well format, the chemical library of 364,131 compounds at Scripps Florida was tested at a single point concentration of 11.9 μM. The assays had outstanding statistics with an average Z'-score of 0.90 +/- 0.03. Of the 1456 hits, 1370 were obtained and rescreened in triplicate demonstrating 1270 confirmed hits. Due to the high number of confirmed hits, an orthogonal assay was devised to try to eliminate potential false positive hits. This orthogonal assay quantified the production of AMP as measured by antibody binding using fluorescence polarization as the readout. The confirmation rate was 43.7%, yielding 599 hits. Based on bioinformatics analysis to select a broad diversity of chemical structures, a subset of 249 compounds was tested in the subsequent dose response assays against the MetRS. The luminescence assay confirmed 137 compounds with activity <10 μM and the fluorescence polarization assay confirmed 96 compounds. Representative compounds from twelve different structural clusters are currently being tested in follow up assays to assess specificity on the trypanosome over the human MetRS analogs, to measure growth inhibition on *T. brucei* cultures, and to explore the potential for hit-to-lead drug development.

1022

ROLE OF NK CELLS (CD56+ CD8+) IN THE PATHOGENESIS OF CUTANEOUS LEISHMANIASIS BY *LEISHMANIA BRAZILIENSIS*

Sara T. Passos, Tais Campos, Lucas P. Carvalho

Federal University of Bahia, Salvador, Brazil

Patients with cutaneous leishmaniasis (CL) by *Leishmania braziliensis* has exacerbated T lymphocyte activation with production of cytokines such as IFN-γ and TNF and high levels of TNF-α associated with disease severity. Recently, attention has been described to the role of CD8 + T cells to deleterious inflammatory response observed in these patients. The role of cytotoxicity in defense mechanism in CL or pathogenesis in humans is not well understood. The main objective of this study is to determine the contribution of NK cells (CD56 +) and NK (CD56 + CD3- CD8+) to immunopathology observed in patients with LC. Using flow citometry and citotoxicity assay as methods to answer our questions, our data showed that the majority of the cells that are positive to grazime and perforin and they are NK cells. This population of NK express CD8 (CD8+ CD56+CD16+ CD4- iNKT-CD3-) and the frequency of these populations are increased in patients with CL compared to healthy subjects. Our preliminary data most interesting demonstrated that the majority of CD8+ T cells in lesions of patients with CL are actually NK cells (CD56 +) expressing CD8+. A sub-population characterized as being CD56+CD3+CD8+ when stimulated with soluble antigen of *Leishmania* (SLA) to verify the ability of these cells to produce IFN- γ had a frequency of 14.3%. In conclusion, this new population described here as CD56+CD8+ is important to citotoxicity in CL patients as a cell responsible to kill infected macrophages.

1023

A SELECTIVE AND IRREVERSIBLE BTK INHIBITOR, IBRUTINIB (PCI-32765) SUPPRESSES A TH2 RESPONSE AND INCREASES RESISTANCE TO CUTANEOUS *LEISHMANIA MAJOR* INFECTION

Gayathri Natarajan, Jason Dubovsky, Natarajan Muthusammy, Amy Johnson, John Byrd, Abhay Satoskar

Ohio State University, Columbus, OH, United States

Treatment for leishmaniasis, in addition to being expensive and highly toxic, is ineffective due to rising drug resistance. Hence, chemotherapies that modulate host immune response towards a leishmanicidal Th1 response are required. In this study we show that Ibrutinib (PCI-32765), a drug shown to be effective in Phase I/II lymphoma trials, is capable of modulating a Th1 dominated immune response during cutaneous leishmaniasis caused by *Leishmania major*. *L. major* infected BALB/c mice treated with Ibrutinib had similar lesion progression compared to vehicle treated *L. major* infected mice until six weeks of infection and subsequently developed smaller lesions from week-6 to week-9 post-infection. This is corroborated by significantly lower parasite burdens in the footpad lesions of week-9 Ibrutinib treated mice compared to footpad lesions from vehicle treated mice. Draining lymph node cells from week-9 Ibrutinib treated mice stimulated with *L. major* antigen produced significantly lower concentrations of IL-4, IL-10 and IL-13 compared to vehicle treated mice while ratios of IFN-γ:IL-4, IFN-γ:IL-10 and IFN-γ:IL-13 were significantly higher in Ibrutinib treated mice compared to vehicle treated mice. Serum IgG1 antibody titers at week-8 post-infection were significantly lower in Ibrutinib treated mice compared to vehicle treated mice while IgG2a titers were similar between the two groups. Thus, our studies indicate that Ibrutinib (PCI-32765) administration suppresses Th2 dominated immune response in *L. major* infection and enhances resistance. This drug requires exploration as a therapeutic agent against leishmaniasis.

1024

COMMUNITY BASED APPROACH TO COMMUNITIES WITH HIGH PREVALENCE OF *ANCYLOSTOMA DUODENALE, STRONGYLOIDES STERCORALIS* AND ANEMIA IN NORTHERN ARGENTINA

Adriana Echazu[1], Ruben Cimino[1], Gladys Paredes[2], Luis M. Arias[3], Viviana Heredia[2], Liliana Caropresi[2], Silvana Lopez[4], Eliana Guillard[5], Eugenia Socías[5], Alejandro J. Krolewiecki[6]

[1]Instituto de Investigaciones en Enfermedades Tropicales - Universidad Nacional de Salta, San Ramon de la Nueva Oran, Salta, Argentina, [2]Hospital J.D. Peron, Tartagal, Salta, Argentina, [3]Ministerio de Salud Pública de la Provincia de Salta, Salta, Argentina, [4]Sistema de Atención Primaria de la Salud (APS) de la Provincia de Salta, Tartagal, Salta, Argentina, [5]Fundacion Mundo Sano, Buenos Aires, Argentina, [6]Consejo Nacional de Investigaciones Cientificas y Tecnicas (CONICET), Buenos Aires, Argentina

Soil-transmitted helminth (STH) infections are the most prevalent neglected tropical diseases, causing anemia, malnutrition and negative consequences in growth and cognitive development of children. Among STH, *Strongyloides stercoralis* (Stst) deserves special consideration due to difficulties in diagnosis and the use of ivermectin for treatment. The aim of this study was to describe the prevalence and morbidity of STHs through a cross-sectional study and the usefulness and feasibility of a community based MDA program integrated to the public primary health care system in two Wichii aboriginal communities (Kilometro6 and Lapacho) in Tartagal, northern Argentina. A statistically representative group using the household as the unit of randomization was selected for surveillance. Single stool samples were analyzed with four methods: sedimentation, McMaster, Agar plate and Harada-Mori. Hemoglobin and antibodies titers against Stst using ELISA-NIE were measured. Single dose albendazole and ivermectin were used for treatment from March to December 2012. The study population included 2289 individuals, 157 had their stool analized. STH prevalence was 47% for hookworms, 13% for Stst and 2% for *Ascaris lumbricoides*. The cumulative prevalence of STH was 55%. Hookworms were all *Ancylostoma duodenale*. Stst seroprevalence was 50%. Anemia prevalence was 55%. Calculated coverage achieved 79%. Tartagal is an area of high prevalence of STH infections where preventive antihelminthic chemotherapy is indicated twice a year. Anemia is a severe public health problem due its high prevalence. The inclusion of ivermectin is justified by the prevalence of Stst, which was detected more frequently by serology. Pharmacovigilance revealed adequate safety of the drug regimen. Community treatment in a house-to-house approach is useful, particularly for risk groups such as preschool-aged children and women of reproductive age. The strategy of integrating the antihelminthic treatment to the primary health care system was successfull in achieving the acceptance of the comunity and high coverage.

1025

POTENTIAL IMPACT OF IMPROVED HELMINTH DIAGNOSTICS ON CONTROL AND ELIMINATION EFFORTS

Michael B. Arndt[1], Grace John-Stewart[1], Barbra A. Richardson[1], Benson Singa[2], Lisette van Lieshout[3], Jaco J. Verweij[4], Laura R. Sangaré[1], Loice W. Mbogo[5], Jaqueline Naulikha[2], Judd L. Walson[1]

[1]University of Washington, Seattle, WA, United States, [2]Kenya Medical Research Institute, Nairobi, Kenya, [3]Leiden University Medical Centre, Leiden, Netherlands, [4]St. Elisabeth Hospital, Tilburg, Netherlands, [5]University of Washington/Kenyatta Hospital, Nairobi, Kenya

The successful implementation of MDA programs can decrease helminth burden and prevalence within communities. Given that WHO guidelines for treatment are based on microscopy screening to determine prevalence, it is important to consider the impact of other testing strategies in these populations. Traditional microscopy methods used to detect helminth infections have limited sensitivity compared with real-time multiplex PCR. However, this improved test performance may modify the ability to detect associations between helminth infection, risk factors, and clinical outcomes. This cross-sectional study was nested within an RCT conducted at 3 sites in Kenya. We performed microscopy and PCR for the stool detection and quantification of *Ascaria lumbricoides*, *Necator americanus*, *Ancylostoma duodenale*, *Srongloides stercoralis*, and *Schistosoma* species. We utilized regression to evaluate associations between potential risk factors or laboratory outcomes and infection as detected by either method. Of 307 adults surveyed, 61(19.9%) and 21(6.8%) were positive for one or more helminth species by PCR and microscopy, respectively ($p<0.001$). PCR-detected infections were associated with farming (RR 1.57, 95% CI: 1.02, 2.40), communal water source (RR 3.80, 95% CI: 1.01, 14.27), and lack of primary education (RR 1.54, 95% CI: 1.14, 2.33), whereas microscopy-detected infections were not associated with any risk factors under investigation. Microscopy-detected infections were associated with significantly lower hematocrit and hemoglobin (means of -3.56% and -0.77 g/dl) and a 48% higher risk of anemia (RR 1.48, 95% CI: 1.17, 1.88) compared to uninfected. Such associations were absent for PCR-detected infections unless infection intensity was considered. Infections diagnosed with either method were associated with increased risk of eosinophilia (PCR RR 2.42, 95% CI: 1.02, 5.76; microscopy RR 2.92, 95% CI: 1.29, 6.60). Differences in helminth diagnostic test performance alter associations between infection, risk factors, and clinical outcomes. As control efforts move to targeted populations and as global efforts shift from control to elimination, it is important to consider the potential implications of using improved diagnostic technologies for helminth infection. These findings suggest that while more sensitive diagnostics identify more infections overall, they may lead to the detection of a higher proportion of clinically less important infections.

1026

THE INTERRUPTION OF THE TRANSMISSION OF SOIL TRANSMITTED HELMINTHS BY PERIODIC MASS CHEMOTHERAPY OF SCHOOL AGED CHILDREN: TRANSMISSION THRESHOLDS AND THE SIGNIFICANCE OF NON-RANDOM CONTACT WITH INFECTIVE STAGES

James Truscott[1], Deirdre Hollingsworth[2], Roy Anderson[1]

[1]Imperial College London, London, United Kingdom, [2]University of Warwick, Coventry, United Kingdom

Previous analyses have highlighted the importance of understanding age-structured mixing to estimate the likely impact of school-based deworming programmes. However, there is no framework for identifying which aspects of the epidemiology of the parasite in the population and the implementation of the treatment program are most important in determining the bounce-back rate of infection following treatment and the opportunities for breaking the transmission cycle. We analyse an age-structured mixing model, in which school-aged children and adults contribute differently to the deposition and acquisition of infective material in the environment and school-age children are subject to periodic chemotherapy. Using a combination of newly derived analytical expressions and numerical simulation, we identify five key parameter groupings that determine the bounce back rate, the effective reproductive number and the point at which the transmission cycle is broken. We show how the bounce-back time (and therefore the critical treatment frequency) is influenced by the relative intensity of infection in the different age groups, the treatment coverage in school-aged children, the extent to which adults and children are exposed to each other's output of infective stages, the relationship between frequency of treatment and the timescale of the survival of infective stages in the environment. This work further illustrates the central importance of parasite sexual reproduction in the context of periodic chemotherapy. Previous work has shown that the effect of worm mate availability has a negligible effect on the undisturbed parasite population, but the pulsed nature of periodic treatment means that its impact can be considerable. Failure to include sexual reproduction in models can lead to significant underestimates in bounce back times and in efficacy of a given treatment regime. We discuss the implications of these findings for the design of treatment programs in different epidemiological settings.

1027

RISK FACTORS FOR SOIL-TRANSMITTED HELMINTH INFECTIONS DURING THE FIRST THREE YEARS OF LIFE IN THE RURAL TROPICS: ANALYSIS OF A BIRTH COHORT

Stefanie Menzies[1], Alejandro Rodriguez[2], Martha Chico[2], Carlos Sandoval[2], Nely Broncano[2], Fabian Salazar[2], **Philip Cooper**[1]

[1]Laboratorio de Investigaciones FEPIS, Pontificia Universidad Catolica del Ecuador, Liverpool School of Tropical Medicine, Quininde and Quito, Ecuador, United Kingdom, [2]Laboratorio de Investigaciones FEPIS, Quininde, Ecuador

Soil-transmitted helminths (STH) infect more than 2 billion humans worldwide, causing significant morbidity in children. There are few data on epidemiology and risk factors for infection in pre-school children. To investigate risk factors for infection in early life, we analysed data collected prospectively in a birth cohort in tropical Ecuador. A total of 2,404 children were recruited at birth and followed up to 3 years of age with periodic collection of stool samples that were examined for parasites using modified Kato-Katz and formol-ether concentration techniques. A questionnaire to collect data on social, demographic, and environmental risk factors was administered to the child's parent at the time of birth. The outcome was defined as the detection of any STH infection during the child's first 3 years of life. STH infections were observed from 7 months of age and prevalence increased with age, with 35.3% of children having at least one STH infection to age 3 years. *Ascaris lumbricoides* was the most common STH infection (present in 28.5% of all infections) followed by *Trichuris trichiura* (16.8%), *Strongyloides stercoralis* (1.1%) and hookworm (0.7%). Independent risk factors for any STH infection in multivariate logistic regression were: Afro-Ecuadorian ethnicity (vs. Mestizo, adj. OR 1.93, 95% CI 1.54-2.42, P<0.001), low maternal educational level (illiterate vs. secondary, adj. OR 1.94 (95% CI 1.34-2.80, P<0.001), urban residence (vs. rural, adj. OR 1.44, 95% CI 1.15-1.81, P=0.001), household crowding (>3 vs. <3 people/sleeping room, adj. OR 1.40, 95% CI 1.13-1.73, P=0.002), and maternal STH infections (e.g. *T. trichiura*, adj. OR 1.68, 95% CI 1.34-2.10, P<0.001; *A. lumbricoides* infection intensity, >3rd tertile vs. uninfected, adj. OR 3.60, 95% CI 2.44-5.30, P<0.001). Our data show that over a third of children were infected with STH parasites during the first 3 years of life in an Ecuadorian birth cohort. Maternal geohelminth infections, and living in an urban environment in conditions of poverty were most strongly associated with the acquisition of STH infections.

1028

WATER, SANITATION AND HYGIENE-RELATED RISK FACTORS FOR SOIL-TRANSMITTED HELMINTH INFECTION IN URBAN SCHOOL- AND PRESCHOOL-AGED CHILDREN IN KIBERA, NAIROBI

Caitlin M. Worrell[1], Stephanie M. Davis[1], Ryan E. Wiegand[1], Gerard Lopez[1], Kennedy Odero[2], Leonard Cosmas[2], Sammy M. Njenga[3], Joel M. Montgomery[2], LeAnne Fox[1], Sharon L. Roy[1]

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

Urban slum dwellers have limited access to city services, including water, sanitation, and hygiene (WASH). WASH factors can affect risk for soil-transmitted helminth (STH) infections, which disproportionally affect school-aged (SAC) and preschool-aged children (PSAC), but further characterization is needed to identify potential interventions. Households containing a PSAC (6-59 months) or SAC (5-14 years) were randomly selected from those enrolled in CDC's International Emerging Infections Program, a population-based surveillance system in the Kibera slum in Nairobi, Kenya. Data collection included a household questionnaire and environmental assessment for WASH risk factors; stool specimens were tested for STH ova by the Kato-Katz method. WASH risk factors were tested for associations with STH infection using univariable and multivariable Poisson regression. STH prevalence among the 201 PSAC and 475 SAC meeting inclusion criteria was 40.8% and 40.0%, respectively. According to WHO/UNICEF water and sanitation ladder classifications, 3.1% of households reported piped water on premises versus 96.9% another improved water source, and 2.3% reported improved sanitation facilities versus 87.7% shared, 6.2% unimproved, and 2.3% open defecation. STH infection was significantly associated with household toilet located off premises (PR=1.33; p<0.05), while always treating water (PR=0.81; p=0.04), clean towel use during hand drying (PR=0.58; p<0.01), finished household floor material (PR=0.76; p=0.01), in-home electricity (PR=0.70; p<0.01), and 10 meter increases in household elevation (PR=0.89; p<0.01) were protective for STH infection. On multivariable analysis, STH infection was significantly associated with treating water usually versus always (aPR=1.5; p<0.01), while finished household floor material (aPR=0.76; p=0.02) and 10 meter elevation increases (aPR=0.90; p<0.01) were protective against infection. The Kibera population faces gaps in water availability and sanitation quality; several modifiable risk factors exist that could be suitable targets for STH control.

1029

RANDOMIZED PLACEBO-CONTROLLED TRIAL OF THE EFFICACY OF MEBENDAZOLE POLYMORPHS IN THE TREATMENT OF HOOKWORM INFECTIONS

Nipul K. Gunawardena, Balachandran Kumarendran, Nuwani H. Manamperi, Buddhika P. Senarathna, Manori Silva, Arunasalam Pathmeswaran, Nilanthi R. de Silva

University of Kelaniya, Ragama, Sri Lanka

Mebendazole has three polymorphic forms, identified as A, B and C. It has been suggested that unlike polymorph C, A is ineffective in the treatment of hookworm and whipworm infections. A randomized double-blind, placebo-controlled trial was carried out to compare the efficacy of single dose 500 mg tablets of pure mebendazole Polymorph C with those containing a 1:1 mixture of Polymorphs A and C, for the treatment of hookworm infections. All eligible individuals living in 219 households were recruited after obtaining written, informed consent. A single fecal sample was obtained and examined the same day, using the Kato-Katz technique for intestinal nematode infections. Those who were found infected with hookworms were randomized to one of three treatment arms and requested to provide a second faecal sample 10 - 14 days after treatment. This was examined in the same manner as the first. A total of 892 individuals were recruited; 601 provided fecal samples; 214 were found positive for hookworm; 70, 74 and 70 individuals were randomized to treatment arms A (mixture of polymorphs A and C), B (pure polymorph C) and C (placebo) respectively. Follow-up samples were provided by 53, 48 and 49 persons respectively in each treatment arm. The cure rates in the three treatment arms were 28.3%, 18.8% and 16.3% respectively; they were not significantly different from one another. Comparison of fecal egg count reductions (FECR) in the 3 treatment arms (86.1%, 84.5% and -6.6% in Arms A, B and C respectively) showed that both mebendazole formulations performed significantly better than placebo, but there was no statistically significant difference between FECR with the two drug formulations. It is concluded that a single 500mg dose of mebendazole, either as Polymorph C alone, or as a mixture of Polymorphs A and C, has little efficacy in curing hookworm infections. However, both formulations were significantly better than placebo in reducing the intensity of infection, with no statistically significant difference between the two formulations.

1030

MUCOSAL IMMUNE RESPONSES DURING HELMINTH TREATMENT FOR INFLAMMATORY BOWEL DISEASES

P'ng Loke

New York University School of Medicine, New York, NY, United States

Helminth treatment for inflammatory bowel diseases has support from experiments in mouse models as well as clinical studies, but the mechanism of action is unclear. Based on a longitudinal analyses of an individual who self-infected with *Trichuris trichiura* to treat his symptoms of ulcerative *coli*tis, we hypothesize that enhancement of mucosal barrier function by TH2 immunity and IL-22 may improve conditions of ulcerative *coli*tis. In this patient, as well as additional other ulcerative *coli*tis patients, TH22 cells (IL-22+, IL-17-) were reduced in tissues with active inflammation and induced in tissues colonized by worms. We then conducted a trial where we treated macaques suffering from idiopathic chronic diarrhea with Trichuris trichiura, collecting biopsies before and after treatment for FACS analyses. A TH2 response was induced and 4 out of the 5 treated macaques improved their symptoms. We also found reduced bacterial attachment to the intestinal mucosa and identified changes to the composition of microbial communities attached to the intestinal mucosa post treatment. These findings suggest that helminth treatment may restore mucosal barrier functions, reducing overall bacterial attachment to the epithelium, and also altering the communities of attached bacteria. We are currently enrolling patients in a double-blinded placebo controlled trial to further investigate these mechanisms in human subjects, treated with *Trichuris suis* ova (TSO). The trial is designed to characterize mucosal responses to TSO treatment and to distinguish between responders and non-responders to TSO treatment.

1031

THE IMPORTANCE OF CONTEXT: HOW SOCIAL AND ENVIRONMENTAL FACTORS MODIFY THE EFFECT OF HEAVY RAINFALL ON DIARRHEA INCIDENCE

Elizabeth J. Carlton[1], Joseph N. Eisenberg[2], Jason Goldstick[2], William Cevallos[3], James Trostle[4], Karen Levy[5]

[1]Colorado School of Public Health, University of Colorado, Denver, Aurora, CO, United States, [2]School of Public Health, University of Michigan, Ann Arbor, Ann Arbor, MI, United States, [3]Centro de Biomedicina, Universidad Central del Ecuador, Quito, Ecuador, [4]Trinity College, Hartford, CT, United States, [5]Rollins School of Public Health, Emory University, Atlanta, GA, United States

The impact of heavy rainfall on water-borne diarrheal diseases is uncertain. This may be due to important biophysical and social factors that modify its effect. We aimed to estimate the effect of heavy rainfall on diarrhea incidence in northern coastal Ecuador, evaluating whether biophysical and social factors impact vulnerability to heavy rainfall events. Active surveillance for diarrhea was conducted weekly for 39 months in 19 villages. We defined heavy rainfall as one-day rainfall in a seven-day period exceeding the 90th percentile value within the study period. Mixed effects Poisson regression was used to test the hypothesis that prior rainfall, water and sanitation coverage and social cohesion modified the relationship between heavy rainfall and diarrhea incidence. We found prior rainfall and drinking water treatment modified the relationship between heavy rainfall and diarrhea. Heavy rainfall was associated with increased diarrhea incidence following 8-week periods of low rainfall (IRR 1.39, 95% CI 1.03, 1.87) and decreased diarrhea incidence following 8-week periods of high rainfall (IRR 0.74, 95% CI 0.59, 0.92). Drinking water treatment reduced the deleterious impacts of heavy rainfall following dry periods. When 67% percent of households reported drinking water treatment, the risk of diarrhea due to heavy rainfall was null (IRR 1.04, 95% CI 0.70, 1.54). Sanitation, hygiene and community social cohesion did not modify the relationship between heavy rainfall and diarrhea. Heavy rainfall appears to cause diarrhea through contamination of drinking water, and presents the greatest health risk following periods of low rainfall. Interventions to increase drinking water treatment may reduce climate vulnerability.

1032

MICROBIAL SOURCE TRACKING IN RURAL INDIA: UNDERSTANDING HUMAN AND ANIMAL CONTRIBUTIONS TO FECAL CONTAMINATION OF IMPROVED AND UNIMPROVED COMMUNITY WATER SOURCES, STORED DRINKING WATER AND HANDS

Mitsunori Odagiri[1], Alexander Schriewer[1], Stefan Wuertz[1], P.R. Misra[2], Pinaki Panigrahi[3], Marion W. Jenkins[1]

[1]University of California Davis, Davis, CA, United States, [2]Asian Institute of Public Health, Bhubaneswar, India, [3]University of Nebraska Medical Center, Lincoln, NE, United States

To reduce the global diarrhea disease burden, the Millennium Development Goal (MDG) 7c aims to increase access to safe drinking water and basic sanitation. The MDG definition of improved drinking water, however, does not include assessment of microbial safety. Several studies in low-income countries have shown improved drinking water sources are contaminated with feces, and household stored drinking water can have higher levels of fecal contamination than source water due to contact with dirty hands. Identification of fecal pollution sources is necessary to better assess health risks and protect drinking water from high risk sources, especially in areas like rural India where animal and open human defecation occur together. Microbial source tracking (MST) using *Bacteroidales* genetic markers is an emerging approach to determine host contributions to fecal pollution.

The goals of this study were (1) to assess the level of human and livestock animal fecal contamination in improved (public and private tube wells for drinking), unimproved (open ponds) water sources, household stored drinking water and on hands in rural communities in India, using MST based on *Bacteroidales* host-associated markers, and (2) to examine the relative microbial safety of improved and unimproved water sources by simultaneously measuring selected pathogens. In 24 villages in Puri, India, 20-L samples of public (n = 43) and private (n = 41) tube wells and ponds (n = 38), 300-mL samples of household stored drinking water (n = 135), and hand rinses of mothers (n = 136) and children (n = 135) were collected in the monsoon season of 2012. After concentration of bacteria and viruses by filtration, fecal sources were determined using quantitative PCR assays validated in India to measure general, human- and bovine-associated markers. Diarrheal pathogens (rotavirus, adenovirus 40/41 and *Vibrio cholera*) in water sources were also tested via qPCR. An unfiltered portion was analyzed for fecal *coli*form. We present the MST and pathogen detection results and discuss the findings.

1033

A CLUSTER RANDOMIZED CONTROLLED EVALUATION OF THE HEALTH IMPACT OF A NOVEL ANTIMICROBIAL HAND TOWEL ON THE HEALTH OF CHILDREN UNDER TWO YEARS OLD IN RURAL COMMUNITIES IN NYANZA PROVINCE, KENYA

Rachel B. Slayton[1], Jennifer L. Murphy[1], Sitnah Hamidah Faith[2], Jared Oremo[2], Aloyce Odhiambo[2], Tracy Ayers[1], Allison C. Brown[1], Robert E. Quick[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Safe Water and AIDS Project, Kisumu, Kenya

Diarrheal diseases are responsible for 17.4% of deaths among children <1 year old globally. Although poor hygiene is an important contributing factor to this disease burden, promoting and sustaining effective handwashing is challenging. Handwashing with soap has been shown to prevent up to 53% of diarrheal, respiratory, and skin infections in young children in developing countries. To address this problem, an innovative technology was developed that consists of reusable hand towels with antimicrobial properties that are non-toxic and stable over time. We conducted a cluster-randomized, longitudinal study to evaluate the impact of the towels on child health. We selected enumeration areas (EAs) that were accessible year round, and randomized them into intervention and control groups. We conducted a baseline survey of mothers and then gave 4 towels to intervention households with handwashing education and handwashing education alone to control households. We made biweekly home visits for 1 year to ask mothers about diarrheal diseases, acute respiratory infections (ARIs), and skin infections in children <2 years old. We tested post-handwashing hand rinse samples of a random sample of 20% of mothers for *Escherichia coli*, an indicator of fecal contamination. At the end of the study, we tested a 50% sample of the towels for *E. coli* contamination. Of 449 enrolled households, 369 (82%) completed >75% of biweekly visits and were included in analysis. At baseline there were no significant differences between intervention and control households. During 8,555 total home visits, enumerators observed at least 3 towels in over 80% of intervention households. There were no statistically significant differences between intervention and control children in rates of diarrhea (1.45 vs 1.47, P=0.99), ARI (1.36 vs 1.50, P=0.57), or skin infections (1.86 vs 1.69, P=0.55) per hundred person-visits. There were no significant differences in post-handwashing hand contamination between intervention and comparison participants and 67% of towels were contaminated with *E. coli*. Antimicrobial hand towels became contaminated with use over time, and did not improve hand hygiene or prevent diarrhea, ARI, or skin infections.

1034

CHOLERA AT THE CROSSROADS: THE ASSOCIATION BETWEEN ENDEMIC CHOLERA AND NATIONAL ACCESS TO IMPROVED WATER SOURCES AND BASIC SANITATION

Benjamin L. Nygren, Anna J. Blackstock, Eric D. Mintz

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

Following major improvements to water and sanitation infrastructure, developed countries have been free of epidemic and endemic cholera for many decades. Most developing countries have made progress in increasing access to improved water and sanitation, but in many countries, cholera persists. The Millennium Development Goals include reducing by half the proportion of the world's population without sustainable access to safe drinking water and basic sanitation. We investigated the association between endemic cholera and national coverage with improved water and sanitation. We analyzed national-level WHO data on annual reported cases of cholera from 1991 to 2010 and estimates of urban and rural improved water and sanitation coverage from 1990, 1995, 2000 and 2005. We compared different definitions of endemic cholera, including the WHO definition of any indigenous cholera cases in ≥3 of 5 consecutive years and definitions using thresholds of annual counts of 10, 50, 100, 250, 500 and 1,000 cases. We performed logistic regression and generated Receiver Operating Characteristic (ROC) curves to assess the use of water and sanitation coverage levels as predictors of endemic cholera within succeeding 5-year periods. National estimates of access to both safe drinking water and basic sanitation were significant predictors of the occurrence of cholera at $p < 0.05$. Values under ROC curves ranged from 0.67 for water and 0.68 for sanitation using the WHO definition to 0.74 for water and 0.74 for sanitation using a definition of 250 cases per year in ≥3 of 5 consecutive years. An important limitation of the data is that many countries periodically fail to report cholera and almost never report counts of zero. This and other data limitations make it challenging to estimate a threshold value for national access to safe water or basic sanitation above which endemic cholera is no longer likely to be seen; however, enhanced definitions of endemic cholera result in improved sensitivity and specificity estimates.

1035

MODELING THE EFFECT OF WATER, SANITATION AND HYGIENE AND ORAL CHOLERA VACCINE IMPLEMENTATION IN HAITI

Isaac Chun Hai Fung, David L. Fitter, Rebekah H. Borse, Martin I. Meltzer, Jordan W. Tappero

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

In 2010, epidemic cholera was introduced to Haiti. Because resources are scarce, decision-makers need to understand the effect of different preventive interventions for ongoing transmission. We built a static model to estimate the potential number of cases averted by water and sanitation improvements (WASH) (i.e., latrines, point-of-use chlorination, and piped water), oral cholera vaccine (OCV), or a combination of both. We allowed for indirect effects and used non-linear relationships between effect and population coverage. We applied 1990-2010 cholera incidence data from Malawi to Haitian demographic data to estimate the potential annual incidence of endemic cholera for a 20-year period in Haiti. We modeled 16 scenarios: six WASH, six OCV, and four that combined WASH and OCV. Over the next two decades, scalable WASH interventions could avert from 57,949 to 78,567 cholera cases, OCV 38,569 to 77,636 cases, and interventions that combined WASH and OCV 71,586 to 88,974 cases. Rate of implementation is the most influential variable, and combined approaches maximized the effect.

1036

KNOWLEDGE, ATTITUDES AND PRACTICES REGARDING CHOLERA, SAFE WATER, SANITATION AND HYGIENE, AND IMMUNIZATIONS PRIOR TO AN ORAL CHOLERA VACCINE CAMPAIGN IN A REFUGEE CAMP - THAILAND, 2012

Heather Scobie[1], Christina Phares[2], Eboni Taylor[1], Kathleen Wannemuehler[1], Nuttapong Wongjindanon[2], Ponchanok RattanadilokNaBhuket[3], **Kashmira A. Date**[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Thailand Ministry of Public Health - U.S. Centers for Disease Control and Prevention Collaboration (TUC), Centers for Disease Control and Prevention-Thailand, Bangkok, Thailand, [3]Thailand Ministry of Public Health (MOPH), Bangkok, Thailand

Cholera is a major cause of morbidity in Mae La refugee camp along the Thailand-Myanmar border; 4 outbreaks occurred during 2005-2012. To complement ongoing safe water, sanitation and hygiene (WaSH) efforts, a preventive oral cholera vaccination (OCV) campaign was conducted in 2013 for all eligible camp residents. In December 2012, before the campaign, we interviewed one respondent in a cross-sectional sample of households (HH) using a standard questionnaire to assess knowledge, attitudes and practices (KAP) regarding cholera, WaSH, OCV and other immunizations, and anticipated OCV acceptability; the survey included HH water testing for residual chlorine and *Escherichia coli*, an indicator of fecal contamination.Among 271 HHs, median HH size was 5.6 persons and 52% had ≥1child aged <5 years. Respondents had lived in the camp for a median of 7.5 (range 1-31) years, median age was 39 (range 15-77) years, 76% were female, 79% were ethnic Karen, and 40% had never attended school. Although 81% of respondents had heard of cholera, only 52% identified watery diarrhea as a symptom. Only 25% respondents said that they treated drinking water to make it safer; of these, 66% boiled water and 9% treated water with chlorine. Soap use for handwashing was reported by 66% of respondents. Overall, 70% of respondents knew vaccines prevented diseases, and 85% of HHs had ≥1 member who ever received vaccines. Only 40% of respondents had heard of cholera vaccine, but 97% were willing to receive free OCV for themselves or their family members. Among stored HH drinking water samples tested, 8% had residual chlorine, and 39% were positive for *E. coli*. Despite frequent recent outbreaks, cholera awareness was low, and safe water and hygiene practices were infrequently used. Knowledge of OCV was low, but anticipated OCV acceptance was high. Preliminary results were used to emphasize cholera disease and WaSH messages during the OCV campaign. A post-campaign survey is planned to assess actual vaccine acceptance and impact of the OCV campaign on cholera knowledge and WaSH behaviors.

1037

CHOLERA VACCINATION CONTRIBUTES TO IMPROVED KNOWLEDGE REGARDING CHOLERA AND IMPROVED PRACTICE RELEVANT TO WATER-BORNE DISEASE IN RURAL HAITI

Omowunmi Aibana[1], Molly Franke[2], Jessica Teng[3], Johanne Hilaire[4], Max Raymond[5], Louise Ivers[1]

[1]Brigham and Women's Hospital, Boston, MA, United States, [2]Harvard Medical School, Boston, MA, United States, [3]Partners In Health, Boston, MA, United States, [4]Partners In Health, St. Marc, Haiti, [5]Partners In Health, Maseru, Lesotho

The cholera epidemic in Haiti has been devastating partly due to underlying weak infrastructure and limited clean water and sanitation. A comprehensive approach to cholera control is crucial, yet influential international policy makers have argued that oral cholera vaccination (OCV) might reduce hand washing and hygiene among vaccine recipients. We aimed to assess the impact of an OCV program on knowledge and health practice in rural Haiti. We hypothesized that such a program, which included education on cholera and hygiene, would improve knowledge and behavior critical for cholera control. We administered surveys on knowledge and practice relevant to cholera and water-borne disease to every 10th household during a census prior to an OCV program in rural Haiti (N=811). We administered the same survey to 518 households randomly chosen from the same region 3 months after the OCV program and compared results using Statistical Analysis Systems (SAS 9.3). Post-vaccination, there was improved knowledge of cholera with significant increase in correct responses on cholera transmission (odds ratio [OR] 1.91; 95% confidence interval [CI] 1.52 - 2.40), preventive methods (OR 1.83; 95% CI 1.46 - 2.30), and means of treating water (OR 2.75; 95% CI 2.16 - 3.50). Relative to pre-vaccination, participants were more likely after OCV program to report always treating water compared to never, sometimes or often (OR 1.62; 95% CI 1.28 - 2.05) and washing hands with soap and water > 4 times a day (OR 1.30; 95% CI 1.03 - 1.64). In pre and post OCV surveys, knowledge of treating water as a cholera prevention measure was associated with practice of always treating water (OR 1.47; 95% CI 1.14 - 1.89). Post-vaccination, knowledge was associated with frequent hand washing (OR 2.47; 95% CI 1.35 - 4.51). Our study revealed that an OCV program in rural Haiti was associated with significant improvement in knowledge of cholera and practices related to water-borne disease. Cholera vaccine can be part of comprehensive cholera control and reinforce, not detract from, other control efforts in Haiti.

1038

CONCOMITANT HOUSEHOLD USE OF LYMPHATIC FILARIASIS INTERVENTIONS IN HAITI

Mathieu J. Poirier, Marie Denise Milord, Ryan R. Hemme, Luccene Desir, Thomas G. Streit

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

Early results from baseline and midpoint household surveys conducted for a multiyear prospective cohort study in the community of Ça Ira, Haiti explored the adoption of interventions against transmission of lymphatic filariasis (LF). Marketing of locally produced Bon Sel salt, co-fortified with iodine and diethylcarbamazine citrate (DEC), has been ongoing throughout the surrounding commune for over a year, and bed net distribution near the community began two years ago to supplement ongoing yearly mass drug administration (MDA) since 2000. The impact of marketing activities on consumption of salt as well as the interaction with bed net use had not previously been examined. Surveys were conducted in November 2011 (n=283) and February 2013 (n=232). Use of bed nets and Bon Sel rose from 48% to 62% and from 0 to 48% of households, respectively. Households reporting the use of at least one bed net were 2.20 (95% CI 1.28-3.79) times more likely to use Bon Sel than those who did not use a bed net in the house. Interviewees' opinions of the benefits of the product were significantly associated with the use of Bon Sel (P<0.001) and those who used Bon Sel were more likely not to wash their salt before consumption (P<0.001). The near-absence of salt washing among those who use Bon Sel supports the value of messaging to ensure the effective delivery of DEC. While those who did not use Bon Sel mostly had no opinion of the product, those who did consume it primarily identified it as being "good for your health". The increase in protective measures against LF occurring concurrently in the same households is a positive development, although households not using either protective intervention may indicate the presence of a persistent filarial reservoir, presenting a possible challenge for the national elimination program. Further study is needed to assess the association of these vulnerable households with non-participation in MDA activities.

1039

FOCAL DISTRIBUTION OF LYMPHATIC FILARIASIS AND OTHER HELMINTHIC INFECTIONS IN LIBERIA

Lincoln Gankpala[1], Lawrence Fakoli[1], Andrew C. Majewski[2], Kurt C. Curtis[2], Fatorma K. Bolay[1], Gary J. Weil[2], Peter U. Fischer[2]

[1]Liberian Institute for Biomedical Research, Charlesville, Liberia, [2]Washington University School of Medicine, St. Louis, MO, United States

Pilot surveys were performed to identify villages suitable for studying the impact of mass drug administration (MDA) on helminthic infections in Liberia. Small cross-sectional surveys of adults assessed prevalence rates for lymphatic filariasis (LF) and other helminthic infections in 45 villages in Grand Bassa, Bong, Maryland and Lofa Counties (total population 1 million). National mapping for LF prior to our study was limited to two localities per county in most cases. Surveys of 564 adults in 11 villages in Grand Bassa County found a mean ICT rate of 3.2% with no microfilaria (Mf) carriers, while the mean ICT rate in 392 adults in 8 villages in Bong County was 2.6% with only 1 Mf carrier. However, the mean *Onchocerca volvulus* (Ov) nodule rate was 26% in the Bong county villages. ICT and Mf rates for 543 adults in 5 villages in Maryland County, Harper District were high (24% and 10%). Ov and schistosomiasis were absent in that area, but soil transmitted helminth (STH) infections were present in 50% of the screened individuals (primarily hookworm and *Ascaris*). Surveys of 1,092 adults in 21 villages in Foya district, Lofa county revealed mean ICT and Mf rates of 16% (range 0-26%) and 9% respectively, while the mean Ov nodule rate was 23% (range 0-47%). Subsequent studies showed high rates of hookworm infection (mean rate 61%) and *Schistosoma* mansoni (mean rate 88%) in Foya District villages. These pilot surveys have revealed distinct distribution patterns for different helminthic NTDs in Liberia. Lymphatic filariasis and onchocerciasis tend to be focally distributed by village or village cluster, while schistosomiasis rates are more uniform within districts. STH infections appear to be endemic throughout rural Liberia, with hookworm being the dominant species. These results provide a rationale for different mapping strategies for different helminthic NTDs in West Africa. It is difficult to justify the current practice of mapping focally distributed LF in just two localities within administrative units with populations that may exceed 200,000 (e.g., counties in Liberia). Two localities per district (with populations in the range of 50-200,000) may be sufficient for STH and schistosomiasis, but we advocate systematic, fine grained mapping for LF and Ov. This could be accomplished by mapping villages according to a grid system to provide data from one locality per 10,000 population.

1040

SEROPREVALENCE AND SPATIAL EPIDEMIOLOGY OF LYMPHATIC FILARIASIS IN AMERICAN SAMOA AFTER MASS DRUG ADMINISTRATION

Colleen L. Lau[1], Kimberly Y. Won[2], Luke B. Becker[3], Wayne D. Melrose[3], Patricia M. Graves[3]

[1]Queensland Children's Medical Research Institute, The University of Queensland, Brisbane, Queensland, Australia, [2]Centers for Disease Control and Prevention, Division of Parasitic Diseases and Malaria, Atlanta, GA, United States, [3]James Cook University, Cairns, Queensland, Australia

Significant progress has been made toward eliminating lymphatic filariasis (LF) from American Samoa. After seven rounds of mass drug administration (MDA) a population-based survey was conducted, and antigen prevalence by immunochromatographic test (ICT) had dropped from 16.5% (N=3018) in 1999 to 2.3% (N=1881) in 2007. In 2011, a WHO recommended Transmission Assessment Survey (TAS) was conducted among 1st and 2nd graders. Of 949 children tested by ICT, two positive cases were identified. Results from the TAS suggested that LF transmission had likely been interrupted and MDA no longer required. However, success of LF elimination programs depends on careful monitoring for potential resurgence of transmission after stopping MDA. Our study aims to provide information to direct future elimination activities by determining seroprevalence, assessing risk factors for infection, and identifying clusters at micro-spatial levels using household locations. Blood samples collected in 2010 from 807 adults in 55 villages were tested for LF antigen and antibody using Og4C3 and Bm14 ELISAs, respectively. Antigenemia was detected in 6 of 807 adults (0.7%, 95% CI 0.3-1.6%, 29-66 years old, 83% male). Bm14 antibodies were detected in 18.2% of samples (95% CI 15.6-21.1%, 18-77 years old, 71% male). Antibodies to Wb123 antigen will also be measured. Two apparent clusters of antigenemic individuals were identified using Kulldorff's spatial scan statistic, with relative risks of >18. Both antigen-positive children from the TAS attended the same school located in one of the clusters. Our study demonstrates the value of geospatial tools for identifying residual foci of infection, potentially allowing more targeted treatment and surveillance to reduce transmission and resurgence risk. Further research is required to identify reasons for clustering of infections (e.g. environmental, vectors, vegetation, or poor MDA uptake). If environmental risk factors are identified, predictive risk maps could be produced with geospatial modelling and used to direct future surveillance and interventions.

1041

PERFORMANCE COMPARISON OF THREE QUALITY OF LIFE INSTRUMENTS IN LYMPHATIC FILARIASIS PATIENTS

Cristina Thomas[1], Kuthaje S. Bose[2], Saravu R. Narahari[2], K. Vivekanada[2], Steven Nwe[1], Dennis P. West[1], Mary Kwasny[3], Roopal Kundu[1]

[1]Department of Dermatology, Northwestern University Feinberg School of Medicine, Chicago, IL, United States, [2]Institute of Applied Dermatology, Kasaragod, India, [3]Department of Preventive Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL, United States

Lymphatic filariasis (LF) is a parasitic infection that affects approximately 120 million people across 81 countries. Characterized by lymphedema and debilitating inflammatory episodes, LF significantly impacts health-related quality of life (HRQoL). As such, HRQoL is a frequently used outcome measure of LF intervention efficacy, despite the lack of a gold-standard LF HRQoL tool. To delineate the strengths and weaknesses of HRQoL tools in the LF population, the relative performances of three HRQoL tools were compared in LF subjects and an age- and sex-matched control group in Kerala, India. All subjects completed the World Health Organization Disability Assessment Schedule (WHODAS 2.0), a generic tool, the Dermatology Life Quality Index (DLQI), a skin-specific tool, the Lymphatic Filariasis QoL Questionnaire (LFSQQ), a disease-specific tool, and a demographic questionnaire. All three tools demonstrated decreased HRQoL in LF subjects as compared to the control group (WHODAS 2.0: 22 vs. 2, DLQI: 6.9 vs. 0, LFSQQ: 23 vs. 1, $p<0.001$). Discriminant validity was best assessed by the LFSQQ. The total WHODAS 2.0 score correlated strongly with the total DLQI score ($r= 0.75$, $p<0001$) and the total LFSQQ score ($r=0.91$, $p<0.001$). Global LFSQQ strongly correlated with total DLQI score ($r = 0.81$, $p<0.001$). Disease stage was not significantly associated with total QoL score of any tool but was weakly associated with the LFSQQ disease burden domain ($p=0.040$) and the DLQI symptoms and feelings domain ($p=0.045$). The DLQI yielded the lowest missing value rate (0%), and the WHODAS 2.0 domains displayed the best internal consistency (mean = 0.85; range = 0.76-0.91), although all tools demonstrated acceptable missing value rates and internal consistency values. Based on the high construct and discriminate validity and acceptable feasibility and internal consistency of the LFSQQ, we recommend use of the LFSQQ in LF HRQoL assessment.

1042

DEVELOPING AN INTEGRATED MICRO-MAPPING TOOL TO INFORM TREATMENT STRATEGIES FOR LYMPHATIC FILARIASIS ELIMINATION IN *LOA LOA* CO-ENDEMIC AREAS OF CENTRAL AND WEST AFRICA

Louise A. Kelly-Hope, Michelle C. Stanton, David H. Molyneux, Moses J. Bockarie

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

The wide distribution of *Loa loa* filariasis through Central and West Africa poses a major obstacle for national programmes scaling up mass drug administration (MDA) for the elimination lymphatic filariasis (LF). Standard drug treatment regimes (including ivermectin) cannot be used due to the risk of severe adverse events (SAEs), especially in medium (20-40%) to high loiasis (>40%) prevalence areas. Many countries lack high resolution LF mapping data and the extent of geographical overlap between these two filarial infections is not known. This study aimed to develop a simple method for potential LF- loiasis co-endemic areas to better classify *L. loa* risk, and to determine if integrated/LF micro-mapping and alternative treatment strategies are required to ensure safe and effective treatment. The geographical information system ArcGIS was used to import and overlap maps, and classify areas accordingly with colour coding. The recent loiasis prevalence map developed from RAPLOA surveys was used a base to define *L. loa* risk at sub-national level according to prevalence i) undefined (white) ii) low risk 40% (red). Based on these classifications it was recommended that sub-national areas with *L. loa* <20% required standard LF mapping and MDA treatment (i.e. green-proceed with standard strategy); areas with *L. loa* 20-40% required integrated LF-loiasis micro-mapping to determine extent of co-endemicity, and potentially alternative treatments (i.e. amber-caution and confirm best strategy); areas with *L. loa* >40% required only LF micro-mapping as high *L. loa* risk was already established and alternative treatments were essential (i.e. red-stop standard strategy). From this, a user-friendly colour coded map of sub-national areas in Central and West Africa was developed to help programmes quantify the number of high risk areas within each country, and areas that may benefit from alternative treatments including vector control, which will allow for more efficient use of financial and human resources.

1043

SEMI-QUANTITATIVE SCORING OF THE IMMUNOCHROMATOGRAPHIC CARD TEST RESULTS FOR CIRCULATING FILARIAL ANTIGENEMIA

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

[1]Institut de Recherche pour le Développement, Montpellier, France, [2]Programme National de Lutte contre l'Onchocercose, Ministère de la Santé et de la Population, Brazzaville, Republic of the Congo, [3]Filariasis and other Tropical Diseases Research Centre, Yaoundé, Cameroon, [4]; Infectious Diseases Division, Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO, United States

The value of scoring filarial antigen test (Binax Now Filariasis card test, ICT) results according to the relative intensities of the test and control lines was evaluated during a survey performed in a village in the Republic of Congo. 134 of 774 tests performed were clearly positive (17.3%), and 11 had questionable results (1.4%). *Wuchereria bancrofti* microfilariae (mf) were detected in night blood smears from 41 of 133 of those with ICT test scores of 1 or higher; mf were not detected in any of 11 slides from people with questionable ICT results. Cuzick's test showed a highly significant trend for higher microfilarial densities in groups with higher ICT scores ($p < 0.001$). Antigen test scores were also significantly correlated with mf counts in individuals. Filarial antigen levels provide an indication of adult worm infection intensity. Our results suggest that semi-quantitative ICT test scores may be useful for grading the intensity of filarial infections prior to treatment and for assessing the impact of treatment on adult *W. bancrofti* worms in individuals and in populations.

1044

EPIDEMIOLOGICAL, CLINICAL, AND LABORATORY EVALUATION OF ONCHOCERCIASIS IN AN AREA OF HIGH PREVALENCE - KITGUM AND LAMWO DISTRICTS, NORTHERN UGANDA 2012

Paul T. Cantey[1], Thomson Lakwo[2], Nicholas Ayebazibwe[3], Shoshana Oberstein[1], Jared Smith[1], Mark Eberhard[1], Anne Moore[1], Vitaliano Cama[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]National Onchocerciasis Control Program, Ugandan Ministry of Heatlh, Kampala, Uganda, [3]The African Field Epidemiology Network, Kampala, Uganda

Onchocerciasis, a neglected parasitic disease, affects at least 37 million people globally. Efforts to eliminate this disease are based on mass drug administration of ivermectin. As part of a project aimed to evaluate tools for measuring the impact of elimination efforts, the authors performed convenience sampling in Kitgum and Lamwo districts in Uganda. The area had a baseline microfiladermia prevalence of 62% and nodule prevalence of 82%. The communities had received ivermectin for less than 5 years, with the last round distributed four months before the study. Risk factor and clinical data were collected on 500 individuals. Blood smears for *Loa* and *Mansonella*, immunochromatographic card tests for filariasis, and skin snips for onchocerciasis were evaluated on-site. Plasma, serum, blood smears, dried blood spots, and preserved skin snips were sent to CDC-Atlanta for further testing. The median age of participants was 32 years (range: 6-92 years); 304 (61%) were female. Onchocercal skin disease was present in 251 (50%) people. At least one nodule was present in 204 (41%) people, with a median of 2 nodules per person (range 1-16). Onchocercal eye disease was present in 40 (8%) people; 27 (5%) had microfilaria in the anterior chamber of the eye. The skin snip was positive for *Onchocerca volvulus* in 127 (25.4%) people. Among those with at least one positive snip, the mean load of microfilaria (MF), determined by averaging the number of MF in both snips, was 9.3 MF per snip (range: 0.5-105.5). On-site tests for other filaria were negative. The OV-16 antibody test was positive in 406 (84%) people. Nine (7%) of those with a positive skin snip were negative for OV-16 antibody. Their ages ranged from 6-60 years. All nine reported taking ivermectin last year. Three had skin nodules. Two had microfilaria in the anterior chamber of the eye. Additional laboratory testing is pending for onchocerciasis and other filarial infections. A better understanding of the performance of the OV-16 antibody test in the African context is needed to ensure proper usage by elimination programs.

1045

MICRO-STRATIFICATION OVERLAP MAPPING OF FILARIAL INFECTIONS IN SOUTH SUDAN

Louise A. Kelly-Hope, David H. Molyneux, Moses J. Bockarie

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

The new Republic of South Sudan has a long history of filarial infections causing onchocerciasis, lymphatic filariasis (LF) and loiasis. Current detailed epidemiological data are scarce due to years of civil conflict, and the national neglected tropical disease (NTD) programme will need to conduct extensive baseline mapping in order to scale up efforts for control and elimination through mass drug administration (MDA). A concern to the expansion of the onchocerciasis and LF programmes is the risk of serious adverse events (SAEs) associated with the use of ivermectin in areas co-endemic with loiasis. To better define these risks, this study examined all past and present filarial data from published literature, and environmental factors associated with their geographical limits and overlapping distributions. Using micro-stratification overlap mapping (MOM) and

geographical information systems (GIS), all three filarial diseases were found to be co-endemic in the south western region, predominately in Western Equatoria State adjoining the border of neighbouring countries. Limited data were available for LF, however, historical onchocerciasis and loiasis distributions from the 1940s-80s significantly overlapped with those from mid-2000s, which were determined from large-scale REMO and RAPLOA surveys. Endemic blinding onchocerciasis caused by *Onchocerca volvulus* was associated with the extensive river system flowing into the Nile River basin, and overlapped with loiasis in the tropical forest - savanna mosaic areas at elevations 400-600 meters, where ferralsols soils were dominant and Chrysops silacea the main *Loa loa* vector. This 'co-endemic hot spot' close to international borders where these diseases also prevail, may pose a significant challenge for the NTD programme in South Sudan. Cross-border coordination and further micro-mapping will be required to define the extent of overlap, identify key risk factors and determine the most appropriate safe and effective treatment strategy, which may include alternative drug regimes and/or integrated vector management.

1046

A CASE STUDY OF RISK FACTORS FOR LYMPHATIC FILARIASIS IN A VILLAGE IN THE REPUBLIC OF CONGO

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

[1]Institut de Recherche pour le Développement, Montpellier, France, [2]Programme National de Lutte contre l'Onchocercose, Ministère de la Santé et de la Population, Brazzaville, Republic of the Congo, [3]Filariasis and Other Tropical Diseases Research Centre, Yaoundé, Cameroon, [4]Infectious Diseases Division, Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO, United States

A study was conducted to identify risk factors associated with *Wuchereria bancrofti* infection in the Republic of Congo. The study was performed in Seke Pembe, a village located approximately 250 km of Brazzaville. Among the 774 subjects aged ≥5 years, 145 (18.7%) had positive filarial antigen tests (ICT card test), and 41 (5.3%) had microfilariae (mf) in night blood smears. The following factors were investigated: sex, age, use of bednets, use of pit latrines, source of water and hunting/fishing activities. Data were analyzed by mixed multivariate logistic regression models. The prevalence of infection (either by ICT or by mf) increased with age up to 16-20 years and remained stable thereafter. The analysis using filarial antigenemia as the dependent variable demonstrated an increased risk for males (odds ratio=2.0, 95% confidence interval [1.3-3.0], p<0.001) and for people who hunt or fish (OR=1.7 [1.1-2.7], p=0.013), and a protective effect of private pit latrines (OR=0.6 [0.4-0.9], p=0.009). Analyses restricted to males showed that those hunting or fishing by night had an increased risk for antigenemia (OR=2.0 [1.1-3.8], p=0.028), and hunting was also a risk factor for females (OR=2.5 [1.1-5.7], p=0.036). Bednet usage was protective for the latter (OR=0.3 [0.1-0.9], p=0.03). There was a strong household effect for females (intraclass correlation coefficient [ICC]: 31%, p=0.0168), but not for males. In the analysis using mf as the dependent variable, males had a higher risk for mf positivity (OR=5.4 [2.1-13.4], p<0.001), private latrines had a protective effect (OR=0.4 [0.1-0.9], p=0.036) and a marked household effect was found (ICC=48.8%, p<0.001). Our results suggest that the effects of these major risk factors are already evident before 20 years of age. Age, sex, and occupation-dependent exposure to mosquitoes seem to be important risk factors for infection with *W. bancrofti*. It appears that males often acquire infections in high transmission areas outside of the village whereas females are infected in areas with lower transmission inside or near the village.

1047

HEALTH IMPACT ANALYSIS OF LYMPHATIC FILARIASIS ELIMINATION VS. ERADICATION SCENARIOS

Chris Stone, Randee Kastner, Peter Steinmann, Nakul Chitnis, Fabrizio Tediosi

Swiss Tropical and Public Health Institute, Basel, Switzerland

The neglected tropical disease lymphatic filariasis has been targeted for eradication by 2020. While great strides have been made to reach this target based on annual rounds of mass drug administration (MDA), a number of endemic countries, many with high levels of prevalence, or co-endemicity with onchocerciasis or loaisis, are behind schedule or are targeting only a small proportion of their at-risk population with their MDA programmes. We present an analysis that compares the health impact expressed as disability adjusted life years lost on a year to year basis over the next half century when current levels of rollout and coverage are maintained (leading to elimination in certain areas, but not affecting disease burden in other areas) to a scenario where a scaling-up to effective levels of coverage occurs everywhere and is maintained until elimination is achieved (eradication). Prevalence of symptomatic disease was calculated using a deterministic model of lymphatic filariasis transmission, Epifil, with parameter values corresponding to an Indian (transmission by *Culex* mosquitoes) and African (transmission by *Anopheles* mosquitoes) setting, while the health burden for 62 countries was calculated using country-specific demographic parameters. Countries were categorized by transmission archetype, depending on the relevant vector genus, initial prevalence, and treatment. A sensitivity analysis accounting for rates of disease progression, demography, and discounting, is presented. Due to the long-lasting nature of symptomatic disease there is a considerable lag between an elimination effort and the accrual of DALYs averted; the consequences of this pattern for cost-effectiveness of interventions will be discussed.

1048

DEVELOPING ALTERNATIVE DRUG DELIVERY STRATEGIES FOR LYMPHATIC FILARIASIS ELIMINATION IN URBAN AREAS IN GHANA

Nana-Kwadwo A. Biritwum

Ghana Health Service, Accra, Ghana

Lymphatic Filariasis (LF) is a significant health problem in developing countries. It causes of permanent disability and undermines the social and economic welfare of affected populations. Mass drug administration (MDA) with ivermectin and albendazole is the strategy for elimination of lymphatic filariasis. MDA in urban areas is a major challenge for elimination programmes. The capital of Ghana, Accra has recorded low MDA coverage over the last 4 years and this study investigated the reasons for this low coverage recorded in urban communities. The objective was to identify the opportunities and barriers for implementing MDA in urban settings in order to develop appropriate strategies for urban MDA. This cross-sectional exploratory and interventional study was carried out in 2011 and 2012. It employed both qualitative and quantitative methods involving household surveys with community members, in depth interviews with health providers and community stakeholders and a transect walk in sampled communities. Urban communities are not uniform and need different but specific strategies to reach its diverse communities and residents. Urban populations are educated and require knowledgeable distributors to respond to pertinent questions such as side effects. All segments are not reached due to inadequate number of community drug distributors (CDDs) deployed while others are engaged to work in communities they are not familiar with. Inadequate social mobilization to create awareness on the importance of participating and complying with the treatment is important. Poor CDD remuneration remains a major challenge. The role of health workers in facilitating MDA in urban areas is central. Recognizing and responding to the needs of varied and sophisticated communities to increase their participation and compliance

in MDA is important together with enhancing social mobilization and improved remuneration of CDDs. Good leadership and supervision are essential to urban MDA activities.

1049

THE IMPACT OF IVERMECTIN ON ONCHOCERCIASIS AND ITS BURDEN OF MORBIDITY AND MORTALITY IN SAVANNAH SETTINGS OF AFRICA

Hugo C. Turner, Martin Walker, Thomas S. Churcher, María-Gloria Basáñez

Imperial College, London, United Kingdom

Mass drug administration with ivermectin has successfully contributed to control onchocerciasis as a public health problem, and in some foci of Latin America and Africa, elimination of the infection reservoir appears to have been achieved with the use of ivermectin alone. However, as control policy shifts from morbidity reduction to elimination at a pan-African scale, it becomes necessary to evaluate the long-term impact of ivermectin both on parasite populations and disease burden. We developed a mathematical model of the dynamics of onchocercal disease by linking previously established associations between infection and morbidity/mortality to output from an onchocerciasis transmission dynamics model. We assessed the long-term impact of mass drug administration with ivermectin on the prevalence and intensity of onchocercal infection and its associated disease burden in savannah areas of Africa, and explored how this impact is influenced by different epidemiological and programmatic scenarios. We assumed that ivermectin efficacy remained unchanged during the programme. Long-term annual ivermectin treatment is highly effective at reducing both morbidity and mortality associated with onchocerciasis, with projected results being relatively insensitive to treatment coverage and compliance. By contrast, overall impact on infection prevalence and intensity is highly dependent on pre-control endemicity, therapeutic coverage and systematic non-compliance, the latter warranting further investigation particularly as programmes advance towards elimination. Our results also indicate that excess host mortality associated with onchocerciasis is a substantial component of its overall disease burden, hitherto underestimated.

1050

SCENARIOS FOR LYMPHATIC FILARIASIS ERADICATION AND THE ASSOCIATED IMPACT ON NUMBER OF TREATMENTS NEEDED

Randee Kastner, Chris Stone, Peter Steinmann, Fabrizio Tediosi

Swiss Tropical and Public Health Institute, Basel, Switzerland

Lymphatic filariasis (LF), a neglected tropical disease causing chronic morbidity, affects an estimated 120 million people. In 2000 the Global Programme to Eliminate Lymphatic Filariasis (GPELF) was established with the goal of eliminating LF as a public health problem within 20 years. While great gains have been made towards controlling LF, elimination by 2020 is unlikely to be achieved in many areas. Scenarios that are more likely to achieve eradication of lymphatic filariasis than the current GPELF strategy are presented that take into account the adaptations necessary to accommodate the disparity between different LF endemic environments. For each scenario, the total number of treatments for Mass Drug Administration (MDA), either administered as albendazole and ivermectin or albendazole and diethylcarbamazine (DEC), is estimated. The analysis utilizes the most current projections of the at-risk population for LF, as listed in the WHO's Preventive Chemotherapy databank, as well as the results of deterministic modeling to predict the number of MDA rounds required to achieve local elimination in given transmission settings. The model takes into consideration different transmission archetypes, including the three different LF species (W. *bancrofti*, B. malayi and B. timori), the primary vectors (*Anopheles*, *Culex*, *Aedes*, Mansoni), and the different MDA protocols (albendazole with DEC or ivermectin). Varying levels of scale up and MDA coverage are considered, with optimistic and pessimistic scenarios developed. The number of treatments required to maintain control or to achieve eradication is compared against each other, as well as against the most recent GPELF's projections and planning documents in order to determine whether a higher level of commitment and resources than currently allocated is necessary to reach eradication.

1051

URBAN TRANSMISSION OF LYMPHATIC FILARIASIS AND OTHER NEGLECTED TROPICAL DISEASES IN URBAN SETTINGS IN TANZANIA

Mbutolwe E. Mwakitalu[1], Mwelecele Malecela[1], E. M. Pedersen[2], F. Mosha[3], P. E. Simonsen[2]

[1]NIMR, Dar es Salaam, United Republic of Tanzania, [2]DBL-Center for Health Research and Development, Copenhagen, Denmark, [3]KCM College, KIlimanjaro, United Republic of Tanzania

Over the last decades there has been considerable increase in urbanization in Africa. Estimates show that by 2030 over half of the population will be living in urban areas. The rapid growth of towns and cities, combined limited economic opportunities, often results in expansion of informal settlements and slums, with favourable conditions for proliferation of vectors and for transmission of many of the neglected tropical diseases (NTDs). This study aims to investigate the epidemiology of lymphatic filariasis (LF) and other NTDs, in two urban settings in Tanzania, Dar es Salaam (metropolis) and Tanga (a smaller city). The study thus addresses issues of high relevance for the control of these infections in urban settings. Pupils from different urban zones of Dar es Salaam and Tanga were screened for infection with LF, urinary schistosomiasis and STH. Nearby communities were examined for LF infection and disease. The human examinations were accompanied by surveys for vector snails and mosquitoes, by questionnaire surveys on hygienic and socio-economic conditions, and by characterization of the environments with regard to water, drainage, sewerage and garbage disposal facilities. The occurrence of LF and other NTDs in the schools and communities (and occurrence of vector mosquitoes and snails) were analyzed in relation to environmental and socio-economic risk factors in the examined urban settings. The usefulness of screening pupils for antibodies to Bm14 for the identification of risk areas for transmission of LF was also assessed. Selected aspects of the findings will be presented. The results from the studies will be used for recommending appropriate surveillance and control measures for LF and other NTDs in urban settings.

1052

EVALUATION OF THE TOURNIQUET TEST FOR THE DIAGNOSIS OF DENGUE INFECTION

Fernando A. Filho[1], Matheus J. Mota[1], Emanuele T. Araújo[1], Natália V. Souza[2], Raissa M. Fontes[2], Dyana A. Silva[2], Jeová K. Colares[1], **Danielle M. Lima**[1]

[1]University of Fortaleza, Fortaleza, Brazil, [2]Federal University of Ceará, Fortaleza, Brazil

Dengue fever (DF) represent a major public health problem worldwide and cause a spectrum of illness ranging from the self- limiting dengue fever to more severe, life-threatening forms of the disease termed dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Brazilian Ministry of Health defines as dengue with complications (DCC) every severe case that does not fit the WHO criteria for DHF. The tourniquet test (TT) has been recommended as a tool to differentiate dengue from other acute febrile illness. The aim of our study was to evaluate the usefulness of the TT among suspected dengue patients. We recruited 30 patients and questionnaire was used to collect clinical and laboratory data. The clinical and nonspecific laboratory data were collected from all patients. The TT was considered positive if 20 or more petechiae were identified. In this study 30 patients with suspected dengue were analyzed, 60% were female and 40% were male. Regarding the clinical form of the disease, 21 (70%) had DF, 5 (16.6%) had DCC and 4 (13.3%) had

DHF. A TT was positive in 33.33% patients, 50% were negative and 16.67% were not performed. With the analysis of blood count, average hemoglobin found was 13.03g/dL, the mean hematocrit was 38.7%, and the average number of leukocytes was 4965.20/mm3. The average of lymphocytes 39.7% and platelets was 113,903mm3. Of DF patients, 62% had hemorrhagic manifestation, 47.6% had leukopenia, 47.6% had thrombocytopenia. Eighty percent of patients had thrombocytopenia and also had hemorrhagic manifestation. All cases of DHF showed warning signs, including persistent vomiting, severe abdominal pain, drowsiness/irritability, fainting and oliguria. In conclusion, DF was the most prevalent classification in this study and all cases of DHF showed warning sign. The TT has been recommended as a tool to differentiate dengue from other acute febrile illness but in this study this method showed small positivity.

1053

EFFECT OF FORMULATION RATIOS AND DOSING SCHEDULES ON THE SAFETY AND IMMUNOGENICITY OF A RECOMBINANT LIVE ATTENUATED TETRAVALENT DENGUE VACCINE (DENVAX) IN HEALTHY ADULT VOLUNTEERS

Gilad S. Gordon[1], Michele Hurliman[1], Bobbi Carlin[2], Joseph Santangelo[3], Richard Rupp[4], Gary Luckason[5], **Jorge E. Osorio**[2], Dan T. Stinchcomb[1]

[1]Inviragen, Fort Collins, CO, United States, [2]Inviragen, Madison, WI, United States, [3]Inviragen, Singapore, Singapore, [4]University of Texas Medical Branch, Galveston, TX, United States, [5]MCR FDN Cardiac Research Department, Loveland, CO, United States

We have been developing a tetravalent, live attenuated dengue vaccine (DENVax) consisting of a molecularly characterized, attenuated DENV-2 strain and three chimeras in which the prM and E genes of the attenuated DENV-2 were substituted with those of DENV- 1, -3 or -4 viruses. Tetravalent formulations of DENVax have been shown to be safe and effective in Phase 1 clinical studies. To further optimize the vaccine, a phase 1b clinical trial was performed to evaluate the safety and immunogenicity of various formulations and administration schedules in healthy, flavivirus negative adults. Two formulations were evaluated which varied in the amount of DENVax-4 virus and included subcutaneous administration of one or two doses on Day 0 with or without one or two doses on Day 90. A total of 115 subjects were evaluated in 5 different groups. To date there have been no serious adverse events. Two subjects discontinued; one for possible arthritis and one for Grade 3 arthralgia/myalgia for one day. The most common systemic adverse events included headache (39%), fatigue (35%), muscloskeletal disorders (35%), and myalgia (27%). The only Grade 3 lab abnormalities were elevated creatine phosphokinase (CPK) values in three subjects following exercise. The vaccine was well-tolerated with mostly mild and transient local reactions. In addition, DENVax induced significant neutralizing antibody responses to all four dengue viruses after one or two administrations. This study highlights the safety and immunogenicity of the different tetravalent DENVax formulations and schedules of administration.

1054

EPITOPE MAPPING OF ENHANCING ANTIBODIES PRODUCED BY THE HUMAN IMMUNE RESPONSE AFTER PRIMARY DENGUE VIRUS INFECTIONS

Ruklanthi de Alwis[1], Katherine L. Williams[2], Michael Schmidt[2], Eva Harris[2], Aravinda M. de Silva[1]

[1]University of North Carolina School of Medicine, Chapel Hill, NC, United States, [2]Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States

Dengue virus (DENV) is a mosquito-borne flavivirus that causes over 390 million new infections globally each year and has become a major public health concern. About 500,000 DENV-infected individuals develop the more severe forms of dengue. DENV exists as four serotypes, named DENV1 through 4. Following a primary infection, individuals produce a mixture of type-specific and cross-reactive antibodies. Pre-existing immunity is sufficient to protect against re-infection with the same serotype, but may increase disease severity during a secondary infection with one of the other three DENV serotypes. A leading theory to explain the higher frequency of severe disease is the antibody-dependent enhancement (ADE) theory, where pre-existing DENV-specific antibodies are thought to bind viral particles and facilitate infection of host cells through Fcγ receptors. Due to the complexity of the humoral immune response, the DENV enhancing antibodies within human polyclonal sera after natural infections has not been well characterized. In the present study, antibodies in DENV-immune human sera were fractionated using recombinant viral proteins, and the role of specific antibody (Ab) populations in DENV enhancement was investigated. Depleted DENV-immune human sera were tested for the ability to enhance DENV in cell culture and in the AG129 mouse model of infection and disease. Enhancement studies with depleted human sera demonstrate that a fraction of enhancing Abs is targeted to the DENV recombinant envelope (rE) protein. The rE protein also contains the fusion loop, to which previous studies have mapped cross-reactive, weakly neutralizing Abs. Many enhancing human monoclonal Abs have also been mapped to the pre-membrane (prM) protein. Competition ADE studies with prM-binding Fab fragments show that prM-binding Abs in human immune sera contribute to ADE of heterotypic serotypes. Further studies are currently being conducted with recombinant pr protein (i.e. the cleaved peptide of prM) to further confirm the role of prM-binding Abs in ADE of heterotypic virus infection.

1055

A STUDY OF DENGUE RISK THROUGH THE LENS OF COMPLEX ADAPTIVE SYSTEMS (CAS) THEORY

Harish Padmanabha

National Socio-Environmental Synthesis Center (SESYNC), Annapolis, MD, United States

Social and ecological changes have caused tremendous variation in the human experience within cities of the Global South, including migration rates, fecundity, social interactions and bio-physical conditions of the residence. All four of these processes have been implicated in the emergence of dengue, but how they interact in the transmission process is unknown, partly because they occur over much larger temporal and/or geographic scales than those at which the mosquito *Aedes aegypti* transmits dengue between people. I present a study of dengue risk through through the lens of complex adaptive systems (CAS) theory and argue that a CAS approach to dengue research can fundamentally transform dengue control in endemic cities and increase the time between epidemics. CAS can be defined as a heterogeneous group of individual agents whose interactions evolve over time based on the outcome of those interactions. A useful way to study CAS dynamics is to frame the system as the interaction between short and long term processes which, in turn, affect the interaction between the system's internal dynamics and external processes. This approach lends nicely to the study of dengue transmission, which is influenced by the interaction between the fast and fine-scale processes of *A. aegypti* life history and slow and multi-scale processes of human life history. I review previous work demonstrating the potential explosiveness of dengue transmission across a group of houses during an inter-epidemic period in Colombia and model its relationship to these larger-scale urbanization processes, using longitudinal data on the mosquito dynamics collected from the same neighborhood and city-wide epidemiological surveillance data. Both the dynamics and socio-ecological drivers of local dengue transmission qualitatively change over time due to interactions between viral introduction from the rest of the city and the human life history processes within the blocks. Due to the spatial aggregation of *A. aegypti* recruitment, vector control efforts will be most effective in areas with increased migration and fecundity, but are ineffective and potentially counterproductive when social interactions facilitate high rates of viral introduction. These findings show that

local dengue transmission has typical CAS properties and highlight the opportunity to use socio-demographic conditions to pattern control strategies in space and time in heterogeneous cities.

1056

REVERSE TRANSCRIPTION-RECOMBINASE POLYMERASE AMPLIFICATION ASSAY FOR RAPID DETECTION OF DENGUE VIRUS

Sazaly AbuBakar[1], Boon-Teong Teoh[1], Sing-Sin Sam[1], Kim-Kee Tan[1], Jefree Johari[1], Mohammed Bashar Danlami[1], Meng-Hooi Shu[1], David Brooks[2], Piepenburg Olaf[2], Nentwich Oliver[2], Annelies Wilder-Smith[3]

[1]Tropical Infectious Diseases Research and Education Centre (TIDREC), University of Malaya, Kuala Lumpur, Malaysia, [2]TwistDx Ltd., Minerva Building, Babraham Research Campus, Cambridge, United Kingdom, [3]Department of Public Health and Clinical Medicine, Epidemiology and Global Health, Umeå University, Umeå, Sweden

Dengue is one of the most important arboviral diseases of human in tropical and subtropical regions of the world. A proper patient management and control of disease spread can be achieved with early and rapid detection of dengue virus (DENV) in patients during the viremic phase. Nucleic acid amplification and detection techniques for dengue diagnosis are available but its application in rural areas of the world where resources can be limited has been challenged due to the need for costly equipment. The recombinase polymerase amplification (RPA) assay is a novel isothermal technology for nucleic amplification and detection without the need for expensive equipment. Here, we evaluated a one-step single-tube reverse transcription-RPA (RT-RPA) assay developed by TwistDx Ltd. for the detection of all four DENV serotypes (DENV-1, DENV-2, DENV-3, and DENV-4). The RT-RPA reagent mixture including the primer set was lyophilized and vacuum-packed to improve the storage stability and to simplify the operating procedure. The diagnostic coverage, sensitivity and specificity of the RT-RPA were evaluated using DENV RNA extracted from DENV infected cell culture supernatant. The RT-RPA assay detected the whole panel of 11 reference DENV strains circulating mainly in the major dengue endemic regions. The RT-RPA assay showed high sensitivity, comparable to the quantitative RT-polymerase chain reaction (qRT-PCR) as it detected up to 10 copies of the viral RNA. The RT-RPA can be completed within 15 minutes. There was no cross reactivity of the RT-RPA with other closely related arboviruses including Japanese encephalitis virus, Chikungunya virus and Sindbis virus. The RT-RPA is a suitable laboratory method for routine dengue diagnosis in rural clinics and field situation where resources are limited.

1057

CHALLENGES FACING DENGUE SURVEILLANCE AND CONTROL - DRAWING ON CASE STUDIES FROM BRAZIL AND INDIA AND THE NEED FOR INNOVATIVE APPROACHES

Ruvandhi Nathavitharana

New York University School of Medicine, New York, NY, United States

The reemergence of dengue has become a major global health issue, responsible for 50-100 million infections worldwide. The changing epidemiology of dengue in recent years has been striking and encompasses the emergence or reemergence of different strains in Central and South America, the explosion of cases in India, and increasing sporadic cases in non-endemic countries due to travel and migration. Challenges for surveillance include the lack of uniformity in case definitions, laboratory capacity and different public health practices between countries. The political repercussions of acknowledging epidemics or true case load are also important considerations. This presentation focuses on lessons learned from cases in Brazil and India, particularly regarding the need for political will and cooperation. Examples include the reluctance to acknowledge the emergence of DENV-4 in Brazil and phylogenetic analyses to identify the origin of the strains. The unwillingness to document the increasing incidence in dengue infections that India is currently facing is also a pressing and timely issue. Greater attention must be paid to surveillance strategies and changing risk factors including climate change and rural-urban shifts. As a result of globalization, our world is increasingly interconnected and there is a call for novel collaborative approaches such as international networks and partnerships to share lab resources or data. Examples of innovation include redesigning vector control strategies to utilize chemoattractant and mechanical mosquito traps, which have been shown to be cost-effective particularly in high-risk areas. There is great hope that efforts to produce an effective vaccine will prove successful but this will undoubtedly raise many questions regarding the need to shift disease control priorities. However key control strategies will remain; such as understanding behavioral risk factors, improving environmental sanitation, raising public heath awareness, producing realistic estimates of the costs involved, developing and maintaining political will and international cooperation. The advent of event based surveillance systems such as ProMED or HealthMap has revolutionized our ability to potentially respond to epidemics. Other innovative surveillance strategies, such as designing early warning systems to provide a lead-time for authorities to adapt preventive measures, also hold great promise.

1058

SEROPREVALENCE OF DENGUE IMMUNITY AMONG MULTIPLE NONHUMAN PRIMATES IN SENEGAL

Derek A. Cummings[1], Mathilde Guerbois[2], Benjamin Althouse[1], Amadou Sall[3], Mawlouth Diallo[3], Diawo Diallo[3], Ousmane Diop[3], Brenda Benefit[4], Evan Simons[4], Douglas Watts[2], Scott Weaver[2], Kathy Hanley[4]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]University of Texas Medical Branch, Galveston, TX, United States, [3]Institut Pasteur de Dakar, Dakar, Senegal, [4]New Mexico State University, Las Cruces, NM, United States

Sylvatic dengue 2 viruses have been isolated from multiple primate species in Senegal. However, the importance of particular species of nonhuman primates to ongoing transmission of dengue in these settings is unknown. Here, we report age-stratified seroprevalence of DENV-2 antibody among African green monkeys (*Chlorocebus sabaeus*), patas monkeys (*Erythrocebus patas*), and Guinea baboons (*Papio papio*) captured over three years (2010-2012) in Kedegou, Senegal. Serum samples were collected from 219 African green monkeys, 78 patas, and 440 baboons that were captured, sedated and then released. The age of each primate was determined using an algorithm that used dental measurements, weight and other anthropometric data. We used this information to estimate the force or hazard of infection of DENV-2 for each species in each season. We find that DENV-2 hazards are high in all species with forces of infection of 0.69 (0.57, 1) for african green monkeys, $\lambda = 0.23$ (0.14, 0.38) for patas monkeys and $\lambda = 0.53$ (0.47, 0.6) for baboons. We also examine heterogeneities by troop and season. Our data suggest sustained, intense transmission of DENV-2 in each of these nonhuman primate species.

1059

EVALUATION OF SAMPLE INTEGRITY USING COMMERCIALLY-AVAILABLE RNA-STABILIZING BLOOD COLLECTION TUBES

Theron Gilliland, Jr., Allison Dauner, Subhamoy Pal, Indrani Mitra, Tadeusz Kochel, Shuenn-Jue Wu

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

Degradation of RNA during specimen transport from collection site to diagnostic facility is a major problem affecting accurate diagnosis of RNA virus infection; speed and cold chain resources are not always available to maintain sample integrity. In this study, we used dengue as a model RNA virus to compare three commercial-off-the-shelf (COTS) RNA-stabilizing blood collection tubes for their ability to stabilize viral RNA in whole blood. We tested these products at conditions simulating low-viremia

samples (102 pfu/mL DENV1 spiked into whole blood) subjected to a loss of cold chain in a tropical environment (37 °C for 0, 8, 16, or 24 hours then restored to room temperature), reproducing possible obstacles in field transport. Using qRT-PCR we found that Biomatrica RNAgard tubes, Tempus Blood RNA tubes, and PAXgene Blood RNA tubes were all able to effectively stabilize dengue viral RNA even when present at low concentrations for up to one month; however, compatibility of these products with qRT-PCR was dependent on the RNA extraction method utilized. Investigations into the use of these products with downstream serological applications demonstrate that they may not be compatible with IgG ELISAs due to high background OD.

1060

ANTIBODY RESPONSE AGAINST SALIVA OF *AEDES AEGYPTI* AND *AE. ALBOPICTUS* IN INDIGENOUS DENGUE PATIENTS IN TAIWAN

Tsai-Ying Yen[1], Kun-Hsien Tsai[2]

[1]Institute of Epidemiology and Preventive Medicine, College of Public Health, National Taiwan University, Taipei, Taiwan, [2]Institute of Environmental Health, College of Public Health, National Taiwan University, Taipei, Taiwan

Dengue is one of the most predominant mosquito-borne infectious diseases. Salivary proteins of mosquitoes containing anti-haemostatic, anti-inflammatory, and immunomodulatory agents that contribute to the success of blood meal. Some of these molecules might involve in the transmission and establishment of pathogens in the hosts, disease progression or epidemic intensity. In Taiwan, *Aedes albopictus* is widespread whereas *Ae. aegypti* is prevalent in the southern counties where most dengue epidemics located. However, due to the frequent transportation on the island, the roles of these two capable dengue vectors played in the dengue epidemiology were yet to be elucidated. In this study, we aimed to investigate human antibody responses against *Ae. aegypti* and *Ae. albopictus* for a better understanding of the roles and functions of mosquito salivary proteins. The results of ELISA showed that dengue patients from southern Taiwan exhibited stronger antibody responses against *Ae. aegypti*, which supported the idea that the mosquito was the major vector responsible for serious dengue outbreaks in southern Taiwan. On the contrary, the readings of antibodies against *Ae. albopictus* were higher in dengue patients of northern Taiwan where only *Ae. albopictus* could be found. Nevertheless, cross-reactions were observed in most sera. Species-specific antigens by applying the pre-absorption procedures before Western blot revealed less than 10 immunogens in the mosquito salivary proteins. After pre-treating sera with salivary gland extracts of *Ae. aegypti*, bands with molecular weights about 44 kD, 35 kD, and 33 kD were absorbed against *Ae. aegypti* while bands with molecular weights about 290 kD, 68 kD, 44 kD, 36 kD, 35 kD, 29 kD, and 15kD were remained against *Ae. albopictus*. The potential applications of these immunogenic salivary proteins that served as biomarkers for exposure to dengue-infected mosquitoes will be discussed.

1061

ADJUSTMENT FACTOR FOR THE REPORTED NUMBER OF DENGUE CASES FROM A PROSPECTIVE COHORT IN PUNTA PRINCESA, CEBU CITY, PHILIPPINES

Frances E. Edillo[1], Maria T. Alera[2], Naomi B. Amoin[1], Yara A. Halasa[3], Francisco M. Largo[1], In-Kyu Yoon[2], Edurado A. Undurraga[3], Donald S. Shepard[3]

[1]University of San Carlos, Cebu City, Philippines, [2]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [3]Brandeis University, Waltham, MA, United States

Dengue illness is one of the eight pervasive infectious diseases in the Philippines. This study aims to provide an empirical estimate of the adjustment factor around dengue reporting based on a cohort study in Punta Princesa, Cebu City, Philippines. Monthly incidence rates (cases/1,000 population) of symptomatic dengue cases were compared between passive surveillance data from the Cebu City Health Department and active surveillance data from a prospective cohort in Punta Princesa. Case definition of symptomatic dengue was based on the Philippine Integrated Disease Surveillance and Response Manual in accordance to revised international health regulations of the World Health Organization. The surveillance phase of the cohort study included weekly SMS, phone call or home visit to check on the health status of 1,000 enrolled volunteers. Volunteers with acute febrile episodes had acute blood samples tested for serotype-specific dengue virus by hemi-nested reverse transcription-polymerase chain reaction (nested RT-PCR) and acute/convalescent blood samples tested by dengue IgM/IgG EIA in March 2012-March 2013. Results for March-October 2012 samples showed an average reporting rate of 13.28% of the confirmed symptomatic dengue cases in Punta Princesa by Cebu City Health Department, leading to an adjustment factor of 7.2 for converting reported cases into estimated actual cases. This is comparable to 7.0 overall expansion (adjustment) factor from a regression equation derived by Undurraga et al from reporting rates across Southeast Asia. This study is relevant in providing an empirical and critical estimate of the expansion factor. It is the first such study in the country, and makes the Philippines one of only 6 countries in Southeast Asia with such an empirical estimate. Such expansion factors are critical is determining a country's economic and disease burden of dengue fever.

1062

DENGUE IN MEXICO: USE OF MULTIPLE DATA SOURCES TO ESTIMATE ECONOMIC AND DISEASE BURDEN

Donald S. Shepard[1], Miguel Betancourt-Cravioto[2], Eduardo A. Undurraga[1], Duane J. Gubler[3], María G. Guzmán[4], Scott B. Halstead[5], Eva Harris[6], Pablo Kuri-Morales[7], Ruth Martínez-Vega[8], Jorge Méndez-Galván[7], Rose Nani Mudin[9], José Ramos-Castañeda[8], Roberto Tapia-Conyer[2], Dengue v2V Under-Reporting Initiative[10]

[1]Brandeis University, Waltham, MA, United States, [2]Carlos Slim Health Institute, Mexico City, Mexico, [3]Duke-NUS Graduate Medical School, Singapore, Singapore, [4]Pedro Kouri Tropical Medicine Institute, Havana, Cuba, [5]Pediatric Dengue Vaccine Initiative, Rockville, MD, United States, [6]University of California, Berkeley, CA, United States, [7]Ministry of Health, Mexico City, Mexico, [8]Instituto Nacional de Salud Pública, Cuernavaca, Mexico, [9]Ministry of Health, Kuala Lumpur, Malaysia, [10]Interlace Global, London, United Kingdom

Despite its control in the 1970s, dengue incidence and severity have increased in Mexico since 1979, currently affecting 23 of 31 states, including the Texas-Mexico border area. Dengue is a reportable disease; Mexico's Ministry of Health (MoH) has promulgated protocols for laboratory confirmation and compiles and disseminates surveillance data. However, as in most countries, surveillance systems are not designed to report every case, so empirical adjustments are needed to extrapolate the overall number of symptomatic dengue episodes. Accurate information about the burden of dengue is needed to set health policy priorities and decisions about disease-control technologies. We merged multiple data sources to estimate (i) total episodes, (ii) costs per episode, (iii) surveillance and vector control costs, and (iv) disease burden using disability-adjusted life-years (DALYs) for 2010-2011. We estimated total episodes of dengue by multiplying reported cases (41,333 episodes in 2010, and 20,548 in 2011) by expansion factors derived from a cohort study in Morelos (2011-2012), and previous empirical estimates. We derived costs per episode from sequential interviews of 36 patients in four major hospitals in Quintana Roo, Morelos, and Tabasco; macro-costing data from two major public hospitals; MoH surveillance data; and previous literature. Indirect costs were based on productivity losses by age. Vector control and surveillance costs were estimated based on MoH data. Preliminary estimates suggest Mexico has about 345,000 dengue episodes (95%confidence interval [CI]: 204,000-434,000) and 135 dengue deaths annually on average. These amount to US$240 million (95%CI: 138-328

million) in illness costs plus US$89 million in federal vector control efforts, totalling US$329 million or US$3.02 per capita (95%CI: 2.08-3.82), and 16,000 DALYs per million population (95%CI: 8,800-22,700). With this study, Mexico joins Panama, Puerto Rico, and Thailand as the only countries or areas worldwide with comprehensive (illness and preventive) empirical estimates of the cost of dengue. These results reaffirm that exploring approaches to control dengue further would be economically valuable.

1063

ECONOMIC COST OF DENGUE VECTOR CONTROL IN MALAYSIA

P. Raviwharmman[1], C. W. Ng[1], M. Dahlui[1], B. Venugopalan[2], Y. A. Halasa[3], D. S. Shepard[3]

[1]University Malaya, Kuala Lumpur, Malaysia, [2]Selangor State Vector Borne Diseases Control Department, Ministry of Health Malaysia, Shah Alam, Malaysia, [3]Schneider Institutes for Health Policy, Heller School, Brandeis University, Waltham, MA, United States

Dengue is endemic in Malaysia, with 46,171 cases reported in 2010 from its population of 28.4 million. The economic cost of dengue is an important policy tool to guide research around new and existing technologies. Vector control against *Aedes* mosquitoes is implemented by the Ministry of Health (MOH), district health offices (DHOs) and local councils (LCs). A previous study derived the cost of dengue illness in Malaysia, but we are not aware of any empirical study of the cost of vector control activities. We estimated public sector dengue control costs as part of a study evaluating the Malaysian dengue vector control program. We analysed costs from the vector control unit of the MOH plus these activities in 7 representative, statistically sampled districts from Malaysia's 160 districts. In each unit selected, both capital and recurrent expenditures were included and both DHOs and LCs were studied in sampled districts. Cost data for the year of 2010 were collected using data collection forms through detailed on-site interviews. Inputs were recorded in a matrix by line item and function. Line items consist of personnel, administrative and storage buildings, vehicles, fumigation equipment, pesticides, personal protective equipment and out-sourcing (fumigation services subcontracted to private companies). Functions consist of inspection, entomological surveillance, fumigation, larvaciding and health education. The analysis extrapolated costs from the sampled districts to the country, estimating both cost per capita and cost per registered dengue case. Costs were converted from Ringgit Malaysia (RM) to US dollars at the 2010 rate (RM3.2=US$1). Preliminary data give mean (± standard deviation) cost per district of aggregate vector control costs ($1.52 ± $0.79 in million), cost per dengue case ($702 ± $138), and cost per capita ($1.70 ± $0.21). Breakdowns by line item showed that human resources was the largest category (58% of costs) followed by pesticides (14%). Breakdowns by function found that fumigation activities had the highest share (36%) followed by inspection of premises (20%), with entomological surveillance as the least costly function (11%). Malaysia is joining the handful of countries worldwide with a comprehensive estimate of dengue costs, combining both illness and prevention. These tabulations are informing the public and stakeholders in at all levels about the economic impact of this disease.

1064

IMPROVING UNDERSTANDING AND SURVEILLANCE OF HUMAN PLAGUE IN NORTHERN UGANDA THROUGH MODELING AND COLLABORATION WITH TRADITIONAL HEALERS

Mary H. Hayden[1], Emily C. Zielinski-Gutierrez[2], Paul S. Mead[2], Apangu Titus[3], Kevin Griffith[2], Andrew J. Monaghan[1], Christine Black[4], Sean Moore[1], Rebecca J. Eisen[2]

[1]National Center for Atmospheric Research, Boulder, CO, United States, [2]Centers for Disease Control and Prevention, Fort Collins, CO, United States, [3]Uganda Virus Research Institute, Arua, Uganda, [4]University of Colorado, Denver, CO, United States

Plague is a highly virulent zoonotic disease that can cause bubonic, septicemic, or pneumonic illness in humans. If recognized early, plague can be treated successfully with inexpensive antimicrobials. Without treatment, more than 50% of bubonic cases are fatal. Although plague occurs worldwide, the overwhelming burden is in rural, impoverished areas of sub-Saharan Africa. During 2004-2009, 97% of the over 20,000 plague cases reported to the World Health Organization were reported from countries in Africa. In northwest Uganda, up to 400 cases are reported annually, with a case fatality rate of nearly 30%. Efforts at the National Center for Atmospheric Research in collaboration with the U.S. Centers for Disease Control and Prevention and the Uganda Ministry of Health have employed meteorological fields and epidemiological data to simulate the spatial and temporal variability of plague in the West Nile region of northwestern Uganda, in order to understand the drivers of the disease, and to identify under-served areas with elevated plague risk. Building on this work, in an effort to reduce the burden of illness in an area with little access to western health care, a training module has been developed and successfully implemented. This module is designed to expand surveillance by bringing together traditional healers and clinical practitioners and is aimed at reducing incidence of plague in rural Uganda. Results from this ongoing, interdisciplinary study will be presented.

1065

ICOPA XIII: THE CHALLENGE OF ORGANIZING FOR THE FIRST TIME AN INTERNATIONAL CONGRESS OF PARASITOLOGY IN A LATIN AMERICAN COUNTRY

Ana Flisser[1], Dolores Correa[2], Fidel de la Cruz Hernandez[3], Guadalupe Ortega-Pierres[3], Patricia Talamas[3]

[1]Universidad Nacional Autonoma de Mexico, Faculty of Medicine, Mexico City, Mexico, [2]Instituto Nacional de Pediatría, SSA, Mexico City, Mexico, [3]CINVESTAV, IPN, Mexico City, Mexico

Mexico City was selected as the site for the next International Congress of Parasitology (ICOPA) to be held in August 10-15, 2014. Previous meetings have taken place in Melbourne 2010, Glasgow 2006, Vancouver 2002, Hokkaido 1998, Izmir 1994, Paris 1990, Brisbane 1986, Toronto 1982, Warsaw 1978, Munich 1974, Washington DC 1970 and Rome 1964. To assure that an inclusive and high quality program is organized, the local scientific committee has asked renowned parasitologists from all over the world and Presidents of National Societies of Parasitology to suggest symposia. The recommended speakers are being selected based on the number of publications in the last five years and the h index using the Scopus database. Up to now we have received and analyzed around 400 speakers distributed in 100 symposia, which gather most parasites related to human diseases as well as veterinary and fish parasites. Areas of research are also very diverse and include immunology, epidemiology, cell biology, molecular biology, ecology and many others. Preliminary data indicate that 54% of potential speakers live in America, 31% in Europe, 8% in Asia, 6% in Oceania and 1% in Africa. Their average number of publications in the last 5 years is 43 and the h index is 25, the latter measures productivity and impact of the published work (i.e. an h=20 indicates that 20 of all articles of an author have been cited at least 20 times). A further aim is to have a green paperless congress, thus we are

making a big effort to organize electronic poster presentations in which each participant will have 30 min for discussion of his work presented as "poster-oral" in a defined program. At the 62nd ASTMH Annual Meeting an up-dated scientific program, including plenary conferences, symposia and poster-oral presentations will be presented.

1066

PATTERNS OF GROWTH FAILURE IN INFANTS IN A RURAL DISTRICT OF PAKISTAN

Asad Ali[1], Tauseef Akhund[1], Najeeb Ur Rehman[1], Fayaz Ahmed[1], William Petri[2], Zulfiqar Bhutta[1], Anita Zaidi[1], Molly Hughes[2]

[1]Aga Khan University, Karachi, Pakistan, [2]University of Virginia, Charlottesville, VA, United States

Malnutrition is major underlying factor in childhood morbidity and mortality in developing countries. Prevalence of chronic malnutrition is high in Pakistan, with a recent study showing 50% of the children being stunted at 18 months of age in a rural district. There is little data regarding patterns of growth failure in children in Pakistan. This pattern is important to study so the highest risk age for growth failure can be identified and appropriate interventions can be planned accordingly. We are conducting a longitudinal observational study of childhood growth monitoring in Matiari, a rural district in Sindh province of Pakistan. We have enrolled 817 infants between 0-1 months of age and are recording their weight and height at monthly intervals. Morbidity data regarding history of upper respiratory tract infections, diarrhea and fever is also being recorded on fortnightly basis. We found that the average weight for age z-score (WAZ) at 1 month was -1.68 for girls and - 1.84 for boys, which decreased further to -1.82 in girls and -2.06 in boys at 6 months of age. At 12 months the mean WAZ increased to -1.75 for girls and -1.99 for boys. Average height for age z score (HAZ) at 1 month was -1.64 in girls and -1.75 in boys, which decreased further to -1.76 in girls and -2.07 in boys at 6 months of age. At 12 months the mean HAZ decreased further -2.3 for girls and -2.65 for boys. This detailed analysis of growth faltering pattern in children will help identify the ages when the children are most at risk for growth failure so appropriate interventions can be planned accordingly.

1067

DENGUE VACCINE INITIATIVE PROJECT: A MULTI-COUNTRY STUDY OF THE ECONOMIC BURDEN OF DENGUE FEVER IN VIETNAM, THAILAND AND COLOMBIA

Vittal Mogasale[1], Jung Seok Lee[1], Jacqueline K. Lim[1], Kang Sung Lee[1], Mabel Carabali[1], Kriengsak Limkittikul[2], Jorge Egurrola[3], Vu Dinh Thiem[4], Aurelio Mejia[3], Pornthep Chanthavanich[2], Bao Ngoc Luong[5], Le Huu Tho[5], Ivan Velez[3], Dang Duc Anh[4], Arthorn Riewpaiboon[2], Jorge Osorio[6], Chukiat Sirivichayakul[2], Brian Maskery[7]

[1]International Vaccine Institute, Seoul, Republic of Korea, [2]Mahidol University, Bangkok, Thailand, [3]Universidad de Antioquia, Medellin, Colombia, [4]National Institute of Hygiene and Epidemiology, Hanoi, Vietnam, [5]Khanh Hoa Health Services, Nha Trang, Vietnam, [6]University of Wisconsin, Madison, WI, United States, [7]Centers for Disease Control and Prevention, Atlanta, GA, United States

Estimation of economic burden of dengue illness is helpful in understanding economic benefits and cost-effectiveness of dengue control strategies and improves evidence base for decision-making. The Dengue Vaccine Initiative is conducting cost of illness (COI) studies on dengue at facility-based fever surveillance sites in Vietnam, Thailand, and Colombia. The COI study estimates the direct and indirect costs associated with lab-confirmed inpatient and outpatient dengue cases in societal perspective. A series of two to three interviews are conducted using a survey instrument to collect the private direct out-of-pocket costs for services, medicines, diagnostics and transport; and indirect cost due to productivity loss among patients and their care takers. At the same time, the study teams collects cost information regarding the treatment provided at collaborating facilities. The total COI per case will be calculated from the sum of out-of-pocket payments, indirect costs, and the facility treatment cost net of any patient co-payments. From October 2011 to March 2013, a total 152 cases in Vietnam, 29 cases in Thailand and 9 cases in Colombia were enrolled in the study. Of the total 143 Vietnamese cases analyzed, 55 were hospitalized while the rest 88 were treated on outpatient basis. The average directs cost was $94 per inpatient and $40 per outpatient. Of the total direct inpatient costs, 30% was spent before visiting the hospital, 24% during the visit and 46% after the hospital visit. The respective direct outpatient costs were 25%, 8% and 67% before, during and after visit. The wage loss in inpatients and their care givers was 12 days and 8 days while outpatients and their care givers had lost wages for 8 days 2 days respectively. In the next step, total COI per case for Vietnam will be estimated and will continue data collection in Thailand and Colombia. The preliminary cost of illness result from Vietnam indicates that dengue illness is associated with substantial out of pocket costs and indirect productivity loss.

1068

INFERRING THE EFFECTS OF COINFECTION ON HUMAN HEALTH: COMBINING RESULTS FROM A SYSTEMATIC REVIEW AND A THEORETICAL MODEL

Emily C. Griffiths[1], Amy B. Pedersen[2], Andy Fenton[3], Owen L. Petchey[4]

[1]North Carolina State University, Raleigh, NC, United States, [2]University of Edinburgh, Edinburgh, United Kingdom, [3]Liverpool University, Liverpool, United Kingdom, [4]University of Zürich, Zürich, Switzerland

Simultaneous infection by multiple parasite and pathogen species is commonplace in humans. Coinfection by parasitic worms alone affects more than a billion people. On top of this are viruses, bacteria, protozoa, and fungal pathogens. How this coinfection affects human health in terms of morbidity and mortality is poorly understood, and how best to treat coinfected individuals is an open question. To address these issues we combined two different, but complementary approaches. First, we systematically reviewed recent publications on coinfection in humans to (i) synthesise the reported impacts of coinfection in humans on morbidity and pathogen abundance, and (ii) characterize any reported interactions among parasites, the parts of the body they infect or consume, and any immune responses they elicit. Most publications reported that coinfections tend to raise pathogen load within coinfected hosts, and exert a greater health burden relative to the effects of single infections. Furthermore, pairs of coinfecting parasites had more potential to interact indirectly via their host, rather than directly, and these indirect effects were more often mediated by shared sites of infection than by shared immune responses. Second, we carried out simulations of a novel theoretical model of two interacting coendemic helminth species in a human population where one species was treated. We found a range of possible outcomes of targeted treatment on the non-target species but, in general, cotreatment of parasites that are natural enemies (those that interact antagonistically with each other) is advisable, whereas species-specific treatments of 'friendly' parasites (those that interact synergistically) confers greater benefits to host health than may currently be appreciated. Taken together, our results suggest that coinfection often, but not always, has deleterious effects on human health, and that knowing and understanding how coinfecting parasites interact may inform optimal treatment strategies for coinfected individuals.

1069

CLINICIAN TRAINING IN SELECT AGENT OUTBREAK RESPONSE IS POTENTIALLY SUSTAINABLE IN RESOURCE LIMITED, EAST AFRICAN COUNTRIES

Edris L. Staples, Matthew S. Chambers, Jaspal Ahluwalia

U.S. Army, Fort Detrick, MD, United States

The United States Army Medical Research Institute of Infectious Diseases (USAMRIID) works closely with The United States Defense Threat Reduction Agency - Cooperative Biological Engagement Program (DTRA-CBEP) and the Ugandan NGO, Makerere University Walter Reed Project, to develop clinician training on early recognition, management and reporting of outbreaks of select agents (e.g., Ebola, anthrax) that is host nation sustainable in the East African Region. Kenyans and Ugandan leaders are (1) effectively engaged in designing and implementing training that improves clinician outbreak response, (2) have the skills and human resources to conduct and sustain the training, (3) committed to devoting time to training without remuneration. Key leaders from Kenyan and Ugandan government ministries, universities and non-governmental organizations met with USAMRIID during a workshop hosted over 3 days in Kampala, Uganda to: Provide constructive criticism of training material presented by USAMRIID, Delineate format and content of the curriculum, Identify and refine specific, measurable, attainable, relevant and timely goals, Develop criteria for selecting trainers-of-trainers, Define the target audience / end-user of the training, Define collaborator roles (consultation, accreditation, implementation, etc.), Establish a timeline for implementing the training, and Begin logistical planning for the first "training of trainers" iteration. Participants filled out Target Asset Maps covering 9 areas of necessary training related activity to inventory their roles and contributions to the training. All of the key objectives were addressed and consensus for a plan and timeline was achieved. Various participants had prior experience and willing to perform in all 9 areas of necessary training related activity; they were committed to working a range of 2-10 hours a week without remuneration. Kenyans and Ugandans consider the training necessary enough to develop their own plan and commit skills and time without remuneration. The training should proceed and funding procured.

1070

EFFECT OF DISTANCE FROM HOUSEHOLD ON UTILIZATION OF CAMPAIGN DISTRIBUTION POSTS DURING AN INTEGRATED CHILD HEALTH CAMPAIGN IN MADAGASCAR

Jodi Vanden Eng[1], Annett Hoppe Cotte[1], Rachelle Desrochers[2], James Goodson[1], Adam Wolkon[1], Marcy Erskine[3], Louise Ranaivo[4], Manisha Kulkarni[5]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]HealthBridge, Ottawa, ON, Canada, [3]International Federation of Red Cross and Red Crescent Society, Geneva, Switzerland, [4]Madagascar Ministry of Health, Antananarivo, Madagascar, [5]Public Health Agency of Canada, Ottawa, ON, Canada

An important concern in planning public health programs is ensuring access to health services and interventions for the target population. This is especially challenging in developing countries with limited resources. Physical distance to health facilities has been associated with accessibility and utilization of routine health services; however, little information is available to assess access to supplemental health services such as child health campaigns. In this study we assessed the effect of distance between household and distribution post on access to interventions during an integrated campaign conducted in October 2007 in Madagascar that delivered measles vaccine, mebendazole, and vitamin A to children 6-59 months of age in 111 districts and long-lasting insecticidal nets (LLINs) to children aged <5 years in 59 districts. Six months after the campaign, a nationwide, community-based survey was performed using a two-stage cluster sampling design. Personal digital assistants equipped with global positioning systems (GPS) were used to collect coordinates of all households in a cluster, select a simple random sample, and enter interview data. In addition, the GPS location of the vaccine/LLIN distribution post was collected for each cluster. Over 51,100 households were mapped and a household member was interviewed in 4302 households from 180 clusters. Most households reported the location of the distribution post to be in their village (76.6% LLIN districts, 62.4% non-LLIN districts). The majority of households reported time to the distribution post to be <30 minutes (80.7% LLIN, 65.2% non-LLIN) or 30 minutes to 1 hour (12.0% LLIN, 22.8% non-LLIN). No significant difference was found in the median distance to a distribution post among children who attended the campaign (326 meters LLIN, 476 m non-LLIN) compared to those who did not (444 m LLIN, 498 m non-LLIN; LLIN p=0.22, non-LLIN p=0.54). In general, these results indicate the distribution posts were well distributed and accessible. GPS is a valuable tool for the evaluation of program activities.

1071

USING PAPER ANALYTICAL DEVICES AND A CELL PHONE TO MEASURE IODINE LEVELS IN URINE

Nicholas M. Myers

Notre Dame, Notre Dame, IN, United States

Urinary iodide (UI) levels are used to assess populations of their risk for iodine deficiency disorders. Most labs measure UI using the Sandell-Kolthoff reaction, in which iodide catalyzes the reaction between arsenic(III) and cerium(IV). The decrease in yellow Ce(IV) is monitored with a spectrophotometer. Prior to analysis, urine samples must be boiled in acidic ammonium persulfate to remove interferents, which requires access to laboratory facilities. A field-friendly paper analytical device (PAD) has been created that could revolutionize the way monitoring agencies test UI. The device requires no power, trained personnel, or instrumentation other than a cell phone. To use the device, the user places 2 drops of urine onto the reaction area and times how long it takes the indicator spot to turn from blue to red. The PAD measures physiologically relevant iodide concentrations at the parts per billion (ppb) level and allows detection of 25 ppb I within 6 minutes. We will focus on several innovations in the implementation of the Sandell-Kolthoff reaction on paper millifluidic devices: 1) We will report a redox indicator that gives much stronger colorimetric results suitable for interpretation by eye or by image analysis programs; 2) We will compare the accuracy and reproducibility of results gathered by video capture with results gathered from a single timed photograph; 3) We will report whether on-paper purification and other analytical strategies can successfully compensate for interferents in urine, eliminating the acidic oxidation step; and 4) Using the principles of life-cycle analysis during the design of the PAD, we incorporated reagents that generate iron oxide nanoparticles in order to entrap the toxic arsenic compounds used in the Sandell-Kolthoff reaction. We will report ICP measurements showing that on-PAD remediation successfully reduces leaching of arsenic from the PAD by over 95%, and that these PADs no longer qualify as toxic waste by the EPA's TCLP assay.

1072

DENGUE VACCINE INITIATIVE PROJECT: A MULTI-COUNTRY STUDY OF THE HOUSEHOLD WILLINGNESS TO PAY FOR DENGUE VACCINES

Jung Seok Lee[1], Brian Maskery[2], Jacqueline KyungAh Lim[1], Kang Sung Lee[1], Mabel Carabali[1], Chukiat Sirivichayakul[3], Jorge Egurrola[4], Vu Dinh Thiem[5], Aurelio Mejia[4], Kriengsak Limkittikul[3], Bao Ngoc Luong[6], Le Huu Tho[6], Dang Duc Anh[5], Ivan Velez[4], Jorge Osorio[7], Pornthep Chanthavanich[3], Vittal Mogasale[1]

[1]International Vaccine Institute, Seoul, Republic of Korea, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Mahidol University, Bangkok, Thailand, [4]Universidad de Antioquia, Medellín, Colombia, [5]National Institute of Hygiene and Epidemiology, Hanoi, Vietnam, [6]Khanh Hoa Health Services, Nha Trang, Vietnam, [7]University of Wisconsin, Madison, WI, United States

As dengue vaccines approach licensure, potential demand for introduction should be examined for policymakers to make evidence-based decision on vaccine introduction. The DVI is conducting multi-country studies on the private willingness to pay (WTP) for dengue vaccines to assess population level perspective of the importance of the vaccine. To estimate household demand and WTP for hypothetical dengue vaccines, we administered a study questionnaire to 400 households in three countries: Nha Trang in Vietnam, Ratchaburi province in Thailand, and Medellin in Colombia. The hypothetical vaccine scenarios were composed of 70/95% effectiveness protective for 10/30 years, respectively. Five pre-assigned prices were determined to reflect local situations. Respondents were asked how many vaccines and for whom they would purchase within their household at randomly pre-assigned prices. The average household size is 4.81 in Vietnam (*N*=400), 5.1 in Thailand (*N*=400), and 4.92 in Colombia (*N*=190). About 28% of the households in Vietnam,and Thailand, as well as 9% of the households in Colombia, have experienced dengue fever. About 90% of respondents have understood the vaccine scenario presented. The raw data show that the average household demand for the hypothetical dengue vaccine is 2.7 at the lowest price and 0.8 at the highest price in Vietnam, 3.9 and 2 in Thailand, and 4.48 and 0.5 in Colombia. These clearly show that there is a higher demand for dengue vaccine at a lower price. Either a Poisson or negative binomial regression model will be chosen as an economic count model depending upon over-dispersion issues in the data from each site. The count model will be used to calculate average WTP and to estimate the average number of vaccines demanded as a function of the price, efficacy, perceptions of dengue severity, as well as household socio-economic characteristics. Non-parametric models will be measured for the youngest child. Both parametric and non-parametric estimates of average WTP provide information regarding the private benefits of vaccination, and enable comparison of household characteristics which may affect purchasing decisions across the countries. Preliminary results indicate that the people are willing to pay for dengue vaccine and the private demand is more likely to be influenced by price than by vaccine effectiveness. More data will be presented at the conference.

1073

A COST-EFFECTIVENESS ANALYSIS OF HUMAN AND PIG VACCINATION STRATEGIES TO REDUCE THE BURDEN OF JAPANESE ENCEPHALITIS IN BANGLADESH

Juliet R. Pulliam[1], Andrew Mirelman[2], Salah Uddin Khan[1], Hossain M. Sazzad[3], M. Jahangir Hossain[4], Repon C. Paul[3], Stephen P. Luby[5], Emily S. Gurley[3]

[1]University of Florida, Gainesville, FL, United States, [2]Johns Hopkins University, Baltimore, MD, United States, [3]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [4]Medical Research Council, The Gambia, Banjul, Gambia, [5]Stanford University, Palo Alto, CA, United States

Japanese encephalitis virus (JEV) is a zoonotic, mosquito-borne *Flavivirus* that circulates among Ardeid birds and can be amplified by pigs. Despite the availability of safe and effective vaccines, Japanese encephalitis (JE) causes an estimated 67,900 human cases each year worldwide and has recently been recognized as an important cause of acute encephalitis and long-term neurological sequelae in Bangladesh. To inform decision-making for future vaccine introduction, we evaluated the cost-effectiveness of human vaccination in three regions of Bangladesh where population-based incidence estimates are available: Rajshahi, Khulna, and Chittagong. We compared the status quo (no vaccination) to a feasible intervention: incorporation of the SA-14-14 2 vaccine into the Expanded Programme on Immunization. The analysis was conducted from a societal perspective and incorporates both direct and indirect costs measured in 2010 US dollars. We also assessed the cost-effectiveness of annual vaccination of domestic pigs to reduce human cases in Rajshahi, where detailed data on pig populations are available. We conducted a 2-way uncertainty analysis of the pig vaccination model because the proportional contribution of pigs to human cases and costs associated with implementation of pig vaccination are unknown. Interventions were considered very cost-effective (VCE) below a threshold equivalent to the *per capita* GDP ($1700) per disability adjusted life year (DALY) averted and cost effective (CE) below a threshold of 3X the *per capita* GDP ($5100). Routine childhood vaccination is expected to be VCE, with a cost per DALY averted of $144 in Rajshahi, $264 in Khulna, and $763 in Chittagong. Probabilistic sensitivity analysis indicates that childhood vaccination has >97% probability of being VCE in Rajshahi and Khulna and 77% probability of being VCE in Chittagong. Vaccination of domestic pigs in Rajshahi would be cost-effective relative to both the status quo and childhood vaccination for all scenarios where pigs are responsible for >30% of human cases and the cost per pig vaccinated is <$10. Our findings suggest that including JEV vaccine into the EPI program would be a cost-effective approach to reduce death and disability from JE in Bangladesh, particularly in Rajshahi and Khulna. The contribution of pigs to human JE in Bangladesh should be quantified to determine if pig vaccination could also be a cost-effective public health strategy.

1074

DENGUE IN INDIA: THE MADURAI CASE STUDY AND NATIONAL IMPLICATIONS

B.K. Tyagi[1], S. Karthigai Selvi[1], C. Vidya[1], N.K. Arora[2], Deoki Nandan[3], Yara A. Halasa[4], Jhanshi Charles[5], C. Mohan Dhanalaxmi[5], Poovazhagi Varadarajan[6], T. Mariappan[1], P. Philip Samuel[1], R. Paramasivan[1], Mukul Gaba[2], Poovazhagi Varadarajan[7], T. Porkaipandian[8], **Donald S. Shepard**[4]

[1]Centre for Research in Medical Entomology, Madurai, India, [2]INCLEN Trust International, New Delhi, India, [3]National Institute of Health and Family Welfare, New Delhi, India, [4]Brandeis University, Waltham, MA, United States, [5]Madurai Medical College, Madurai, India, [6]Madras Medical College, Chennai, India, [7]Institute of Child Health, Chennai, India, [8]Public Health and Preventive Medicine, Chennai, India

Dengue is a notifiable disease in India since 1996, with an annual average of 20,018 laboratory confirmed cases reported from 2006-2012. However,

the true magnitude of dengue burden is poorly understood due to scarcity of diagnostic tests, limited scope of the surveillance system, and other constraints. An accurate understanding of the dengue burden will help estimate the economic cost of this illness, assist policy makers in evaluating the benefit and effectiveness of various prevention and control technologies, and develop a combination of strategies to control dengue. A case study to estimate an adjustment factor to project the true number of dengue cases was conducted in Madurai District in the state of Tamil Nadu. A descriptive inventory of all health facilities or laboratories treating or testing dengue patients in the district was developed. The laboratories were classified by type of dengue test performed and hospitals were stratified by bed capacity. Numbers of suspected and confirmed dengue cases for the years 2009 through 2011 were obtained from the public laboratory for all public ambulatory facilities and a stratified sample of 12 of the 250 hospitals. Dengue cases from hospitals were extrapolated based on the ratio of total beds to beds in sample facilities by stratum. Projected dengue cases from the case study were compared with the officially reported numbers from the district surveillance unit (DSU) to the state level. The state-level estimate was then adjusted for the share of cases tallied at the national level. The DSU reported an annual average of 126 confirmed dengue cases, compared to a projected annual average of 3,324 cases from this case study. On an annual average, the DSU captured 86 (37%) of the 231 confirmed dengue cases treated in public hospitals and 48 (2%) of the 2,167 confirmed dengue cases treated in private hospitals, but none of the 905 laboratory-confirmed ambulatory cases, and none of the 1.3 dengue deaths. For laboratory confirmed dengue cases, the reporting rate at the state level (126/3324) was 3.73% and the expansion factor (3,324/126) was 26.4, and the national expansion factor was 47.1. Applying the national expansion factor to the reported cases suggests that India had an annual average of 943,000 confirmed dengue cases. This case study provides the first empirical estimate of an expansion factor for India, and the country experiences almost a million confirmed dengue cases annually.

1075

DEVELOPMENT OF A SERVICE MODEL FOR MOBILE DATA COLLECTION, CLOUD-BASED REPORTING AND CENTRALIZED DATA MANAGEMENT TO SUPPORT MULTIPLE NTD PROGRAMS

Alex L. Pavluck[1], Rebecca Mann Flueckiger[1], David Dyck[1], Anthony W. Solomon[2], Eric Ottesen[1]

[1]Task Force for Global Health, Decatur, GA, United States, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom

The rapid expansion of mobile networks globally, coupled with the increasing functionality and decreasing cost of mobile equipment allows global health projects to increasingly utilize mobile and web-based technology tools in their work. The development and support for a simple-to-use mobile data collection system running on Android devices, coupled with a cloud-based reporting and data management system will make data collection faster, cheaper, and more accessible to programs wanting to utilize electronic data collection. While many tools exist to collect electronic data, most systems require considerable time and resources to move into production. By building a shared system as a service model we are able to provide servers, personnel, mobile equipment, and ongoing support at low cost to programs. Methods: Using the open source tool, Open Data Kit, we developed a modified survey application to streamline data entry, plus a web-based reporting website to expand online reporting and management of collected data. Over the past two years the LINKS System has been used in over twenty-five projects, surveys have been conducted by nine lead organizations (the largest to date being the Global Trachoma Mapping Project) and have resulted in the successful collection of millions of geo-referenced, easily-accessible data points. In conclusion, using a cloud based centralized system for smartphone data upload and web-based reporting services, we have developed a system that can be deployed on a large scale, across multiple countries, at low cost.

1076

MEASURING IMPACT OF SUPPLY CHAIN INTERVENTIONS USING A DIFFERENCE-IN-DIFFERENCES APPROACH

Savitha Subramanian[1], Megan Noel[1], Yasmin Chandani[2], Sarah Andersson[1], Sophia Magalona[1], Mildred Shieshia[2], Barbara Felling[1]

[1]JSI Research & Training Institute, Inc., Arlington, VA, United States, [2]JSI Research & Training Institute, Inc., Nairobi, Kenya

SC4CCM is a learning project that tests simple, affordable solutions to address unique supply chain challenges faced by community health workers to generate knowledge that can be put into broader practice for supply chain operations, financing, and advocacy. The project developed a Theory of Change (TOC) to serve as a technical framework to guide the monitoring of change, the demonstration of success, and serves as the umbrella framework for country-specific TOCs. The interventions proposed to strengthen community supply chains are based on analyzing the relative strength of the system performance elements in the TOC and their preconditions based on data gathered at baseline in both countries. In Ethiopia, a group training approach was employed to improve supply chain management knowledge, skills, and tools among health workers. In Malawi, two interventions were implemented to improve community level product availability. One used a team approach to create a customer service oriented supply chain, while the other addressed transportation efficiency. To measure the impact of these interventions on product availability over time - a difference-in-differences (DiD) approach was used to subtract the effect of changes in supply chain outcomes in the comparison areas from changes in the intervention areas during the study period. Intervention and comparison groups were matched using relevant characteristics at baseline. Each DiD model was designed to take into account components from country-specific causal pathways in the TOC. The results for both Ethiopia and Malawi interventions did not find significant effects of the interventions on product availability, likely due to overlapping effects of factors introduced after the design of the interventions, affecting product availability in both groups. In the absence of significant DiD results, the TOC provided the project with a robust way to measure the true impact of the interventions and led the project to look deeper into causal pathway indicators to draw out the lessons from the interventions.

1077

IMPROVING COMMUNITY LEVEL SUPPLY CHAIN PERFORMANCE USING TEAM-LED, DATA DRIVEN SOLUTIONS IN MALAWI AND RWANDA

Yasmin Chandani[1], Megan Noel[2], Sarah Andersson[2], Amanda Ombeva[1], Mildred Shieshia[1], Alexis Heaton[2], Barbara Felling[2]

[1]JSI Research & Training Institute, Inc., Nairobi, Kenya, [2]JSI Research & Training Institute, Inc., Arlington, VA, United States

In Malawi and Rwanda, community health workers (CHWs) treat children under five for pneumonia, diarrhea and malaria in their villages. Baseline assessments in 2010 identified gaps in supply chain knowledge, skills, procedures, information and decision making all of which contributed to low responsiveness to stockouts by CHWs and their managers. To address these barriers, SC4CCM partnered with 3 districts in each country to design and implement a series of interventions to first improve knowledge, skills and logistics data visibility, and then to promote a team-led approach to identifying solutions to close the supply chain performance gaps. While each country specific intervention was named and customized to reflect the local context, the team-based approaches had four key elements in common: 1) Teams consisting of CHWs and district and health center (HC) staff; 2) Joint identification of problems, development of plans that include targets for improvement; 3) Mechanisms for using data for performance monitoring and problem solving techniques for developing solutions; and 4) Use of recognition or rewards for motivation. Mixed-method evaluation surveys were conducted in early 2013, consisting of quantitative data collection from 10 districts in each country. In Malawi, 76% of CHWs

and 96% HC staff participated in meetings and over 80% of CHWs and HC staff knew their performance plans and targets,. Key performance monitoring indicators improved as well; reporting rates went from 43% at baseline to consistently above 90% for the six months prior to the survey, and reporting completeness was 90%. In Rwanda, monitoring data results showed 83% of HCs had full team member participation in meetings, 92% of HCs had team members who used the correct tool to identity SC performance problems, and 100% of HCs had teams that completed performance graphs after the last meeting. We concluded, therefore, that providing multi-level teams with a structure and process to identify, monitor and address problems is an effective approach to supply chain performance improvement.

1078

MATERNAL AND NEONATAL MORBIDITY IN SOUTHERN PROVINCE, ZAMBIA

Katherine Semrau[1], Julie M. Herlihy[1], Caroline Grogan[2], Arthur Mazimba[3], Kojo Yeboah-Antwi[1], Reuben Mbewe[4], Lutango Alisheke[5], Davidson H. Hamer[6]

[1]Boston University Center for Global Health and Development, Department of International Health, Boston University School of Public Health, Boston, MA, United States, [2]Boston University Center for Global Health and Development, Boston, MA, United States, [3]Boston University Center for Global Health and Development, Zambia Center for Applied Health Research and Development, Choma, Zambia, [4]Ministry of Health, Lusaka, Zambia, [5]Southern Province Health Office, Livingstone, Zambia, [6]Boston University Center for Global Health and Development, Zambia Center for Applied Health Research and Development, Department of International Health, Boston University School of Public Health, Lusaka, Zambia

There is limited understanding of the prevalence of maternal and neonatal morbidity in Zambia. In the context of a large neonatal survival trial (ZamCAT), we investigated the prevalence of maternal and neonatal health danger signs and associated socio-demographic characteristics. Data on antenatal and postnatal maternal danger signs including fever, vaginal bleeding, convulsions, swelling, severe headache, postpartum hemorrhage and breast pain were collected prospectively during home visits. Reported neonatal danger signs in the first 28 days of life included fever, hypothermia, jaundice, convulsions, omphalitis, diarrhea and difficulty breathing. Data were collected from 27,293 pregnant women and their neonates. 82.4% of the women were married; 10.2% had no formal education and 39.6% had completed primary school. HIV prevalence was 8.9%. Mean maternal age (± SD) was 25.6 ± 6.9 y and women had on average 3.6 ± 2.3 pregnancies. 2.95% had at least one danger sign during the antenatal and postnatal periods; most occurred on day 1 postpartum. The most common maternal danger sign at day 1 was excessive vaginal bleeding (0.64%). Women with at least one danger sign had a higher mean number of previous pregnancies (3.8 ± 2.4 vs. 3.5 ± 2.3 pregnancies, ($p<0.01$) and were older (26.3 vs. 25.6 years, $p<0.01$) . HIV-infected women were 1.52 times more likely (95% CI: 1.24, 1.87) to have at least one danger sign. 2.79% of neonates had at least one danger sign with highest prevalence at day 1 (1.35%), most commonly fever (0.47%) and difficulty suckling (0.63%). In contrast to the maternal health correlates, mothers of children with at least one danger sign had a lower mean number of past pregnancies [3.3 ± 2.1 vs. 3.6 ± 2.4 pregnancies) ($p<0.01$)] and were younger [25.1 vs. 25.7 years, $p<0.05$] compared to mothers of healthy children. Maternal HIV infection had no impact on the prevalence of neonatal danger signs. Danger signs occurred infrequently in pregnant women and newborns. HIV infection increased the risk of maternal morbidity but did not influence neonatal complications.

1079

DEVELOPMENT, VALIDATION AND INTERNATIONAL FIELD PERFORMANCE OF MULTIPLEX REAL-TIME QPCR ASSAY PANELS FOR THE DETECTION OF DIARRHEAGENIC VIRUSES, BACTERIA AND PROTOZOA

Darwin J. Operario[1], Jie Liu[1], Mami Taniuchi[1], Stephen Becker[1], Lalitha Janacki[1], Shihab Uddin Sobuz[2], Sharmin Begum[2], Mamun Kabir[2], Rashidul Haque[2], Michel Dione[3], Jainaba Manneh[3], Martin Antonio[3], Senyael Swai Ndealilia[4], Athanasia Maro[4], Jean Gratz[1], Tayyab Un Nissa[5], Furqan Kabir[5], Adil Kalam[5], Shahida Qureshi[5], Anita Zaidi[5], Pimmnapar Neesanant[6], Sasikorn Silapong[6], Lertsethtakarn Paphavee[6], Ladaporn Bohidatta[6], Carl Mason[6], Eric Houpt[1]

[1]University of Virginia Health System, Charlottesville, VA, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [3]Medical Research Council, Banjul, Gambia, [4]Kilimanjaro Christian Medical Centre, Moshi, United Republic of Tanzania, [5]Aga Khan University, Karachi, Pakistan, [6]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand

With their increased sensitivity and decreased time to diagnosis, PCR-based diagnostics represent a significant improvement over to conventional microscopy, culture-based and ELISA-based methods. In this work, we developed and validated five TaqMan-based real-time qPCR assay panels for the detection of sixteen diarrheagenic pathogens including viruses, *E. coli* and non-*E. coli* bacteria, and protozoa. The validation process for all panels revealed at least five logs of linear range for each gene target with low to moderate CV values in within-run ("repeatability") and between-run ("reproducibility") precision tests. In addition, the assays perform well with stool extracts having varying degrees of inhibition. The assay panels were also evaluated at five international field sites using one of three real-time PCR platforms including the Bio-Rad CFX96, the Corbett/Qiagen Rotorgene, and the Applied Biosystems ViiA 7. Field evaluations were based upon the same linearity, repeatability, and reproducibility measures used during the validation process. While there were site-to-site differences for certain targets, the assay panels performed well over the five field sites, and importantly, similarly between the three real-time PCR hardware platforms. ROC curve analysis comparing the assay panels to conventional methods revealed that the assay panels have good predictive value. Future work will entail defining the Ct values that are predictive of a pathogen being associated with diarrhea versus asymptomatic carriage.

1080

WHY THE PRIVATE SECTOR IS A VITAL PARTNER IN SCALING UP DIARRHEA TREATMENT: LESSONS LEARNED FROM NEPAL, PAKISTAN, BENIN, AND GHANA

Vicki MacDonald, Emily Sanders

Abt Associates, Bethesda, MD, United States

Diarrhea is a leading cause of child deaths worldwide. Secondary DHS analysis of childhood diarrhea practices from 46 countries indicate a significant portion of caregivers from all wealth quintiles seek care from the private sector_ranging from 79% in South Asia and 66% in South East Asia to 50% in Sub Saharan Africa. In Nepal, Pakistan, Benin, and Ghana, USAID's POUZN and SHOPS projects developed public private partnerships to reach private providers and leverage private sector expertise: local manufacturers of quality affordable zinc/ORS assured distribution and marketing to retail providers through existing commercial channels; providers were trained in management with ORS and zinc; commercial advertising firms created compelling demand creation messages; and public sector partners assured an enabling policy environment for private sector engagement. Population-based household surveys were conducted in Nepal (n=3550), Pakistan (n=1713), and Benin (n=392) with caregivers of children under five with diarrhea to explore knowledge and practices following program implementation. All three countries showed increases in zinc use. Affordability, availability of zinc

products and recall of zinc messaging were important drivers of use. In Pakistan, there was a statistically significant association (p<.001) between exposure to a zinc message, either from media or a doctor, and use of zinc. In Benin, logistic regression analysis showed that recalled exposure to zinc messages, speaking to health personnel, seeking treatment from a health provider, and caregiver perceptions of zinc being effective and readily available increased odds of using zinc. In Ghana, mystery client surveys and interviews with providers showed increased knowledge and practices around prescription of zinc and ORS. Private sector sales of zinc tablets rose ten-fold after training and 300% after a media campaign. Together, this data shows the vital importance of engaging and leveraging the private sector in the introduction and scale up of zinc for childhood diarrhea treatment globally.

1081

AN EVOLUTION IN GLOBAL HEALTH INVESTMENTS: MOVING TOWARD SUSTAINABILITY

Deborah Derrick

Friends of the Global Fight Against AIDS, Tuberculosis and Malaria, Washington, DC, United States

Building on incredible progress against HIV/AIDS, tuberculosis and malaria, there is an evolution taking place in the world of global health delivery and financing. Many countries at the center of these epidemics - with the initial emergencies coming under control - are now able to scale up and take on an increasing level of responsibility for the health of their own citizens. The Global Fund to Fight AIDS, Tuberculosis and Malaria was founded more than a decade ago as an emergency response mechanism to address the three deadly epidemics. With approximately $23 billion in funding approved for more than 150 countries, the organization and its partners have helped to save 100,000 lives every month. As a result of the progress made to date - and to ensure that the fight continues in the most effective way possible - the Global Fund is evolving to become a catalyst for a more sustainable response to the three diseases. Friends of the Global Fight Against AIDS, Tuberculosis and Malaria recently completed a review of growing country capacity, analyzing the various ways implementing countries are strengthening health systems, building political will, and increasing domestic health investment. Using secondary research as well as a series of interviews from countries in Latin America, Asia, and sub-Saharan Africa, the resulting report identifies key trends, best practices and progress on the ground. This session, led by Deborah Derrick, President of Friends of the Global Fight Against AIDS, Tuberculosis and Malaria, will: provide an overview of the progress made against the diseases; outline the trends and best practices identified through research; articulate different ways sustainability can be measured - including, but not limited to domestic co-investment; highlight key country examples; and describe the roles of the various funding mechanisms - from multilateral and bilateral institutions to domestic and private sector investment.

1082

CHARACTERIZING HUMAN INTERACTIONS WITH PERIDOMESTIC RODENTS IN THAILAND: A PARTICIPATORY RAPID APPRAISAL

Laura R. Seckel[1], Kanokwan Suwannarong[2], Susan Zimicki[1]

[1]FHI 360, Washington, DC, United States, [2]FHI 360, Bangkok, Thailand

Rodents are carriers and reservoirs of numerous pathogens that pose significant risk to humans, including many emerging diseases. In both urban and rural areas, peridomestic rodents (Family Muridae), such as rats and mice, pose a public health threat because they regularly come into contact with humans and domestic animals, providing opportunities for disease transmission. To prevent the spread of disease from these rodents to humans, feasible and acceptable strategies must be developed to minimize human exposure to rodents and their excrement. However, mitigation strategies will only be successful if they are framed within the context of the perspective, knowledge, skills and resources of a given community. To inform the development of mitigation strategies, we use rapid appraisal and participatory research techniques to characterize peridomestic rodent exposure among men, women and children in four sites in Khon Kaen province, Thailand, where peridomestic rodents are viewed as both a destructive nuisance and a source of food and income. The study characterizes peridomestic rodent exposure in these communities and identifies barriers to and opportunities for mitigating the risk of exposure and captures the continuum of people's experiences with peridomestic rodents - from 'opportunity' to 'mild annoyance' to 'serious threat.' Main themes explored in analysis are the location of peridomestic rodents, signs of peridomestic rodents, types of people who come into contact with peridomestic rodents, risks associated with interactions with peridomestic rodents, seasonal variations in exposure, the effects of peridomestic rodents on communities, perceived health impacts of peridomestic rodents, and mitigation strategies currently practiced in the communities. Factors such as gender, location (urban vs. rural, flood zone vs. non-flood zone), age, and occupation are considered. Based on study findings, several potential mitigation strategies are proposed.

1083

WHO IS AT HIGH RISK OF EXPOSURE TO AN EMERGING ZOONOTIC DISEASE? AN INNOVATIVE APPROACH TO CHARACTERIZING HUMAN-ANIMAL EXPOSURE IN HMONG AND LAO ETHNIC GROUPS IN LAO PDR

Jariseta Rambeloson Zo[1], Khounkham Xaymounvong[2], Phongsavay Chanthaseng[3], Cecile Lantican Lantican[2], Sara Woldehanna[1], Tula Michaelides[1], Susan Zimicki[1]

[1]FHI 360, Washington, DC, United States, [2]FHI 360, Vientiane, Lao People's Democratic Republic, [3]Department of Communicable Control Diseases, Vientiane, Lao People's Democratic Republic

A recent increase in emerging infectious diseases (EIDs) -- most of which are zoonotic - has been attributed to an increase in contact between humans and their domestic animals and wild animals. To help inform the design and implementation of interventions to reduce the transmission of EIDs, our study aimed to provide estimated rates of human exposure to different animals, quantify the risk of emerging infectious diseases, and identify populations or subgroups with particularly high rates of exposure. The population-based household survey among Hmong and Lao adult men (n=292), women (n=292), boys (n=203), and girls (n=189) was conducted in Khamkeut district, Bolikhamxay province, Lao PDR in March/April 2013. Independent samples were obtained for men and women aged 18-50 in each ethnic group; up to one girl and one boy aged 10-14 years of age were selected from every adult household that had children who met the age criteria. Animals of interest were wild animals particularly likely to carry zoonotic viruses -- bats, rodents, and primates - although the survey also collected information on other types of wild animals to which people were exposed. Selected domestic animals (pigs and chickens) were used as controls. A human-animal-interface assessment framework was developed to describe the relationship between different transmission routes, types of animal and human activities, and social and environmental factors influencing the emergence of zoonotic infectious diseases. Key indicators of contact related to different transmission routes were weighted to create an overall index of exposure to a specific animal. Simple and multiple logistic regressions were used to identify factors associated with being at "higher risk" of exposure to an EID. The main types of animal humans have contact with, and seasonal variations in specific human activities that put humans in contact with animals were assessed, and the frequency of exposure to animals and intensity of exposure were measured for each subgroup. This study is among the first to examine and quantify comprehensive human exposure to animals. It will help us understand how exposure to animals is mediated by social and environmental factors and identify interventions that might reduce risk of emerging infectious diseases in Lao PDR.

1084

RECOGNIZING MATERNAL EMPOWERMENT FOR IMPROVING UNDER-FIVE CHILD DIARRHEA IN INDIA

Poulomy Chakraborty, John D. Anderson, IV, Richard D. Rheingans

University of Florida, Gainesville, FL, United States

India accounts for one fifth of global burden of childhood rotavirus mortality. With mothers being the primary care givers; their characteristics significantly influence child health. However, India has a gender inequality index of 0.62 ranking 129th (UNDP,2011) indicating a gap in female household decision-making. This analysis aims to understand the association between maternal empowerment (ME) and (i) the likelihood for a child to get diarrhea (ii) predicted rotavirus vaccination (RV) coverage nationally and in 6 regions (north, central, east, west, south, northeast). Estimates are based on National Family Health Survey III (2006) representing 19,138 children. ME Index comprising 11 variables (household decision making roles in social, economic, health domains) is created using Principal Component Analysis and categorized using quartiles. Indian Government plans to introduce a 2 dose RV hence the 2 doses of Diphtheria, Pertussis, Tetanus (DPT) vaccination are used as proxy. Logistic regression is used to predict associations, while adjusting for maternal education, settings, maternal employment and household wealth relative to the lowest ME quartile. Significant association is found between the highest quartile of ME with decreasing odds of child diarrheal incidence, (OR: 0.79, 95% CI 0.66, 0.94) and increasing odds of DPT2 coverage (OR: 1.29, 95% CI: 1.12, 1.47), nationally. Regionally, the odds of RV coverage is found to increase significantly in north (2nd quartile - OR: 1.72, 95% CI: 1.24, 2.40; 4th quartile - OR: 1.82, 95% CI: 1.25, 2.64), west (4th quartile - OR: 1.75, 95% CI: 1.11, 2.78) and south (3rd quartile - OR: 2.20, 95% CI: 1.50, 3.23 and 4th quartile - OR: 4.04, 95% CI: 2.65, 6.16). The odds of diarrheal incidence is found to decrease significantly in northeast (3rd quartile - OR: 0.35, 95% CI: 0.17, 0.72 and 4th quartile - OR: 0.48, 95% CI: 0.26, 0.90) and in south (4th quartile - OR: 0.53, 95% CI: 0.33, 0.85). Thus, region specific ME interventions in India could be highly impactful to improve child health and thereby reduce diarrheal incidence and mortality.

1085

BURULI ULCER OUTREACH EDUCATION: AN EXEMPLAR FOR COMMUNITY BASED TROPICAL DISEASE INTERVENTIONS

Mark Nichter[1], Eric Koka[2], Arnaud Amoussouhoui[3], Ferdinand Mou[4], Evaristus Mbah[4], Koin Tohnain[5], Paschal K. Awah[6], Micah Boyer[1]

[1]University of Arizona, Tucson, AZ, United States, [2]Noguchi Memorial Institute for Medical Research, Legon, Ghana, [3]Centre de Dépistage et de Traitement de l'Ulcère de Buruli, Allada, Benin, [4]Fairmed Cameroon, Yaoundé, Cameroon, [5]University of Yaoundé, Yaoundé, Cameroon, [6]Centre For Population Studies and Health Promotion, Yaoundé, Cameroon

Buruli ulcer is a neglected disease caused by infection of subcutaneous tissue with *Mycobacterium ulcerans* that can be treated effectively by a regimen of antibiotics if identified early, but which requires surgery if identified in its latter stages. In this presentation, we describe an innovative approach to BU education outreach as a means of increasing community awareness about the disease toward the end of early identification, decreased treatment delay, enhanced treatment adherence, and decreased treatment drop-out. The shortcomings of top down education approaches have been well documented. Education is never introduced into vacuum and needs to address existing perceptions/misperceptions, practices, and ways of knowing in a culturally sensitive manner. We describe the development and piloting of a BU education program based on formative research carried out in three West African Countries. The program utilizes a question:answer format and addresses issues of concern to both health staff and local populations encompassing BU signs/symptoms and ways of distinguishing BU from other common diseases, BU progression, perceptions of possible causes of BU, and treatment. The program is driven by a power point presentation that may be easily updated when new questions arise, and translated into local languages at community events. It also incorporates images explaining all actions taken by clinic staff in identifying and managing BU, and time series images of stages of BU healing so the public knows what to expect from treatment. Testimonials of former patients attest to quality of care such that the education program is driven by hope instead of fear. We present the steps taken in developing and piloting the intervention, the results of a three country evaluation, and data on people attending screening camps following the education programs. The program is offered as an exemplar for other tropical disease education programs.

1086

DO PROVIDER PERCEPTIONS OF MALARIA CASE MANAGEMENT PRACTICES MATCH ACTUAL PATIENT BEHAVIOR AT PRIVATE SECTOR DRUG RETAIL SHOPS IN NIGERIA?

Eric Schatzkin, Naomi Beyeler, Anna De La Cruz, Dominic Montagu, Jenny Liu

University of California San Francisco, San Francisco, CA, United States

In Southwest Nigeria, even though most patients seek care for malaria from private sector pharmacists and patent and proprietary medicine vendors (PPMVs), little is known about provider treatment and patient care-seeking behavior for malaria case management. In 2012, we conducted qualitative research with pharmacists and PPMVs to assess provider perceptions of malaria case management. We then conducted a quantitative study of patient treatment-seeking behavior among patients purchasing anti-malarials from these types of retailers in Oyo State. Using a mixed methods approach, this study assesses the extent to which there is congruence between provider perceptions of customer demand and malaria case management preferences, and actual patient behavior at private sector pharmacies and PPMVs. Transcripts from 20 provider interviews and focus groups discussions were coded to identify domains of provider perceptions of malaria case management. These domains are then compared to survey data eliciting actual patient care-seeking behavior for malaria. We find that provider perception of malaria prevalence is much higher than confirmed malaria prevalence among pharmacy and PPMV customers (confirmed malaria prevalence was less than 5%). Providers perceived themselves as the primary malaria diagnosticians for their customers, but patients overwhelmingly reported diagnosing themselves (over 90%). Providers also expressed a desire to use rapid diagnostic tests for malaria, but patients reported a lack of trust for pharmacists or PPMVs to administer them. Providers perceived trust to be an important factor for where patients choose to seek care, which accords with reported customer preferences. Both providers and patients incorrectly associate many symptoms with malaria. These findings reinforce the importance of private sector drug retailers' role in malaria case management, but indicate that targeted interventions, including those to patients, may be necessary to change diagnosis and treatment behavior.

1087

IMPACT OF DISPARITIES ON THE POTENTIAL HEALTH IMPACT OF ROTAVIRUS VACCINATION IN NIGERIA: RESULTS OF AN INDIVIDUAL-BASED RISK MODEL

John D. Anderson, Richard Rheingans, Julia Showalter

University of Florida, Gainesville, FL, United States

Rotavirus diarrhea is a leading cause of child mortality in low-income countries, which could be reduced by new vaccines. We developed a model of rotavirus vaccine impacts for Nigeria, where mortality from diarrhea is high, and there are large disparities in vaccine access. We examined disparities in risk and vaccine access; how they affect vaccine impact, and how reducing disparities could improve health gains. We used the 2008-9 Nigeria Demographic and Health Surveys to develop an

individual model of diarrheal mortality and rotavirus vaccination. Child-level effectiveness was estimated based on whether children received DPT vaccinations, vaccination timing, illness age, and vaccination efficacy. A susceptibility index was developed from estimates of nutritional vulnerability and likelihood of diarrheal treatment to assess individual mortality risk for each child. The 20% of children with highest risk had 56% of the overall burden. Children in the poorest 20% of households had an estimated 35% of overall burden but only 8% of total vaccination effectiveness and 14% of the vaccination benefit. The estimated health cost due to these disparities is 46 times higher for children in the poorest as compared to richest quintile. Geospatial analyses display concentrations of risk and low vaccine effectiveness hotspots, located especially in the northeast and northwest regions of Nigeria. Disparities in coverage and effectiveness reduce vaccine impact, especially for the most vulnerable children. Reducing disparities in coverage could increase impact two-fold. Improving our understanding of disparities mechanisms could inform programmatic and policy decisions, improving health gains of vaccine introduction.

1088

SOCIOECONOMIC AND ENVIRONMENTAL DETERMINANTS OF CHILD DIARRHEA IN THE BRAZILIAN AMAZON

Athos M. Brana, Thasciany M. Pereira, Breno M. Delfino, Fernando L. Branco, Antonio C. Martins, Saulo A. Mantovani, Humberto O. Guzman, Rhanderson G. Campos, Cristieli S. Oliveira, Pascoal T. Muniz, **Mônica da Silva-Nunes**

Federal University of Acre, Rio Branco - AC, Brazil

Diarrheas are an important aspect of child health, especially in developing countries. The Brazilian Amazon is still an underdeveloped region, although some financial and social investments have been made by the Brazilian government throughout the decades. We analyzed the prevalence of diarrhea in children under 5 years of age in 2003 (n= 200) and 2010 (n = 377) in the same municipality in the Brazilian Amazon. A census was performed with mother of children living in the urban area of Assis Brasil, in the State of Acre, in the Western Brazilian Amazon, in order to determine the prevalence of self-referred diarrhea in the previous 15 days. Differences in prevalence were tested with Chi-square test or Fisher test. Diarrhea prevalence in 2003 was 34.5% in this age strata, decreasing to 18.2% in 2010 ($p < 0.001$, Chi-square test). In 2010, prevalence of diarrhea was higher in children between 12 and 24 months of age ($p = 0.01$) and in those attending baby care ($p = 0.063$). Socioeconomic variables associated with infant diarrhea were belonging to a household receiving governmental financial help ($p = 0.006$), indigenous ethnicity ($p = 0.047$), having a mother with very low income ($p = 0.062$), low schooling ($p = 0.01$) or who did not have a job in the previous 30 days ($p = 0.05$). Environmental factors were also associated with diarrhea. Living in houses made of wood ($p=0.022$), that were frequently flooded ($p = 0.039$) or that had an open sewage system ($p=0.06$), using a latrine ($p = 0.01$), not having piped water in the household ($p = 0.001$), electric power ($p = 0.011$), cooking stove ($p = 0.003$) or refrigerator ($p = 0.023$). The source and quality of drinking water was also a very important factor associated with diarrhea. Diarrhea was more frequent in children that did not have access to treated drinking water ($p = 0.004$) or mineral water ($p = 0.011$). Interestingly, adding chlorine, filtering or boiling the drinking water did not decrease the prevalence of infant diarrhea. In 2003, no variables were associated with diarrhea, probably to insufficient sampling. In this part of the Brazilian Amazon, infant diarrhea prevalence is high and still associated with socioeconomic features, quality of drinking water and household environment, which suggests an infectious etiology for it. Health promotion and investments in education and basic sanitation are still needed.

1089

ANEMIA IN PREGNANCY, BIRTH OUTCOMES AND CHILD HEALTH IN KENYA

Julia L. Finkelstein[1], Odada Sumba[2], Fredrick Opinya[2], Paul Baresel[3], Arlene Dent[4], Saurabh Mehta[1], Rosemary Rochford[3]

[1]Cornell University, Ithaca, NY, United States, [2]Kenya Medical Research Institute, Kisumu, Kenya, [3]State University of New York Upstate Medical University, Syracuse, NY, United States, [4]Case Western Reserve University, Cleveland, OH, United States

Anemia is common during pregnancy, particularly in resource-limited settings such as Sub-Saharan Africa. This study was conducted to examine the prevalence of anemia in pregnant women, and the relationship between maternal anemia, adverse perinatal outcomes, and child health in Kenya. Participants were 191 pregnant women enrolled in a prospective cohort study of perinatal health in Kisumu, Kenya. Binomial regression was used to estimate risk ratios and 95% confidence intervals, and multiple linear regression models were used to examine associations between maternal hematological status (hemoglobin, anemia (Hb<11.0g/dL), severe anemia (Hb<8.5g/dL) and adverse pregnancy outcomes, including low birth weight (<2,500 grams), preterm birth (<37 weeks gestation), and neonatal anthropometry. The average gestational age at enrollment was 20.8 weeks and the mean body mass index was 22.4 kg/m2. 50.5% of women had anemia and 7.9% had severe anemia at baseline, with an average hemoglobin concentration of 10.9 (± 1.7) g/dL. The mean birth weight of the children born to the participating women was 3,198.2 (± 437.0) grams with an average gestational age at birth of 39.2 (± 4.3) weeks. The incidence of low birth weight was only 5%; however, 18% of all children and 25% of the male newborns were stunted at birth (WHO length-for-age Z-score<-2). Maternal anemia was not associated with increased risk of low birth weight or other pregnancy outcomes. In conclusion, a majority of the women in this study had anemia during pregnancy; however, it was not associated with increased risk of adverse pregnancy outcomes. The incidence of low birth weight was lower than the national average though one in five children were born stunted.

1090

MALARIA AND NEGLECTED TROPICAL DISEASES IN PREGNANCY: RESULTS FROM A PROSPECTIVE PERINATAL COHORT STUDY IN KENYA

Saurabh Mehta[1], Julia L. Finkelstein[1], Odada Sumba[2], Fredrick Opinya[2], Paul Baresel[3], Arlene Dent[4], Rosemary Rochford[3]

[1]Cornell University, Ithaca, NY, United States, [2]Kenya Medical Research Institute, Kisumu, Kenya, [3]State University of New York Upstate Medical University, Syracuse, NY, United States, [4]Case Western Reserve University, Cleveland, OH, United States

Malaria and neglected tropical diseases remain a major threat to maternal and child health, particularly in Sub-Saharan Africa. In this analyses, we examined the prevalence of malaria and neglected tropical diseases in pregnant women, and their association with adverse perinatal outcomes in Kenya. Participants were 191 pregnant women enrolled in a prospective observational cohort study of perinatal health in Kisumu, Kenya. Binomial regression models were used to estimate risk ratios and 95% confidence intervals and linear regression models were used to examine associations between maternal infections and adverse pregnancy and infant outcomes. Parasitic infections (33%), including malaria (21%), pathogenic protozoan (20%), and helminth (11%) infections were common in pregnant women at baseline. Maternal malaria infection was associated with a five-fold increased risk of low birth weight (<2,500 grams; p=0.02); similar significant associations were observed for the outcomes of underweight (WHO weight-for-age Z-score < -2), wasting (WHO weight-for-length Z-score < -2), and BMI-for-age z-scores. In a novel finding, maternal malaria predicted a 1.44 cm decrease in head circumference at birth, equivalent to a reduction in head circumference-for-age by 1.14 (p<0.01). Pathogenic protozoan infections including *Entamoeba histolytica* and

Giardia lamblia were associated with a two-fold increase in the risk of stunting (p<0.01). Multiple intestinal parasitic infections were associated with a four times greater risk of stunting in male infants, compared to infants born to women with monoinfection or no infection (p<0.01). In summary, malaria and neglected tropical diseases are common in pregnancy in this population in Kenya. These infections severely compromise infant growth *in utero* and are associated with lower achieved weight, length, and head circumference at birth. A comprehensive approach to target infectious diseases in pregnancy is needed to reduce the risk of adverse perinatal outcomes in resource-limited settings.

1091

EPIZOOTIC MONITORING OF CLASSICAL SWINE FEVER IN POPULATION OF WILD BOARS IN THE TERRITORY OF UKRAINE

Oleg Nevolko[1], Mykola Sushko[1], Serhiy Nychyk[2], Mykola Sytiuk[2], U. Muhtu[2]

[1]SSRILDVSE, Kiev, Ukraine, [2]IVM, Kiev, Ukraine

We have conducted a retrospective epizootic, serologic, molecular and genetic monitoring of classical swine fever (CSF) in pigs in population of wild boars within Ukraine. When performing serologic monitoring, we used 3919 blood serum samples taken from boars shot off in hunting areas in 24 administrative oblasts of Ukraine during 2001-2009 hunting seasons. ELISA test-systems from IDEXX have been employed for the detection of antibodies to CSF virus in blood sera. 383 biological samples were studied to detect CSF virus RNA with Amplisens real-time PCR kits. The monitoring resulted in the revealing of 400 samples of positive blood sera. An average seroprevalence index was calculated at the level of 8.3 %. The seroprevalence indexes per hunting season were: 16.7 % in 2001, 36.4 % in 2002, 28.0 % in 2003, 7.9 % in 2004, 13.2 % in 2005, 8.8 % in 2006, 13.9 % in 2007, 6.0 % in 2008, 8.0 % in 2009, 6.4 % in 2010, 3.5 % in 2011, and 5.9 % in 2012. The serology study results indicated the presence and circulation of CSF agent in the wild boars population. It should be pointed out that the last CSF outbreak in wild boars was registered in Cherkassy oblast of Ukraine in 2002. RNA of CSF virus was detected by real-time PCR in one of 383 studied samples of biological materials. This sample was taken from a wild boar shot off in Kyiv oblast and analyzed in SRILDVSE. The obtained results of epizootic, serologic, and molecular-biological monitoring allow concluding the presence of CSF virus in the population of wild boars in the territory of Ukraine. This information allowed us to hypothesize that wild boar serve as a reservoir for CSF virus in Ukraine.

1092

FOLDSCOPE: ORIGAMI-BASED PRINT-AND-FOLD PAPER MICROSCOPE

James S. Cybulski, James Clements, Manu Prakash

Stanford University, Stanford, CA, United States

Here we describe Foldscope, a fully functional microscope printed and folded out of paper. Foldscope utilizes the principles of origami to implement bright field, multi-fluroscence, polarization, dark-field and projection microscopy. Foldscopes are manufactured in a roll-to-roll process where all the optical components including lenses, apertures and illumination electronics are directly printed on paper. Novel optical components including spherical GRIN lenses and integrated micro-apertures are also introduced. Panning and focusing are implemented in a single paper stage using a flexure mechanism. The entire microscope can be assembled in minutes, is extremely light (paper), packs in a completely flat configuration in a very small volume, operates with no external power, can be dropped from a 5-story building or stomped upon by a person, and can be incinerated if required. We hae established several potential applications of foldscope in ultra low cost "use and throw" microscopy for field diagnostics of diseases including malaria, chagas, giradiasis. Costing less than a dollar, Foldscopes can bring microscopy out of the lab and into the hands of citizen scientists enabling large-scale hands-on life science education opportunities.

1093

PREVALENCE OF CHRONIC KIDNEY DISEASE IN AFRICA: A SYSTEMATIC REVIEW AND META-ANALYSIS OF POPULATION BASED STUDIES

Samuel A. Abariga, Bruce Kupelnick

Tufts Medical Center, Boston, MA, United States

Globally, Chronic Kidney Disease (CKD) has now been increasingly recognized as a major health concern. It is widely known as a major cardiovascular risk multiplier and can rapidly progress to end stage kidney disease requiring expensive treatments not limited to dialysis and kidney transplant. The burden of CKD in the developed world has been documented often in the medical literature; however, its prevalence in Africa remains unknown and unstudied. We performed a systematic review and meta-analysis of all the published evidence on the prevalence of CKD in population-based studies in Africa that use the standardized definition from the National Kidney Foundation Kidney Disease Improving Global Outcomes (KDIGO) practice guidelines. We conducted a comprehensive literature search of 11 electronic databases through April 2013 with no language restriction. We used the combination of various keywords relevant to CKD in our literature search and systematically reviewed published epidemiologic studies on the prevalence of CKD in Africa. We used a random effects model based on inverse variance method to estimate the pooled prevalence. We identified 644 potentially relevant citations and retrieved 64 full articles after title screening. Six studies published between 2008 and 2012 in five countries enrolling a total of 3936 participants whose mean age ranged from 28 to 54 years were included ultimately in the review. To estimate Glomerular Filtration Rate (eGFR) 2 studies used the Modification of Diet in Renal Disease (MDRD) equation. Three studies used both the MDRD and the Cockcroft-Gault (C-G) equation for eGFR while the remaining study assessed CKD using albumin creatinine ratio. The pooled prevalence of CKD by the MDRD vs. C-G estimating equations was 5% (95% CI: 3% to 8%) versus 16% (95% CI: 7% to 25%). Available evidence suggests that the MDRD equation tends to underestimate GFR in healthy individuals; in contrast, the C-G tends to overestimate. In conclusion, few population-based studies on the prevalence of CKD in Africa exist. Despite the difference in various estimating equations of GFR, the current body of evidence suggests significant prevalence of CKD in Africa, and further epidemiologic studies may be warranted for more accurate documentation.

1094

DENGUE IN INDIA: THE COST OF ILLNESS

Donald S. Shepard[1], Yara A. Halasa[1], B.K. Tyagi[2], Vivek S. Adhish[3], Deoki Nandan[3], K.S. Karthiga[2], C. Vidya[2], Mukul Gaba[4], Narendra K. Arora[4]

[1]Brandeis University, Waltham, MA, United States, [2]Centre for Research in Medical Entomology, Madurai, India, [3]National Institute of Health and Family Welfare, New Delhi, India, [4]INCLEN Trust International, New Delhi, India

Between 2006 and 2012 India reported an annual average of 20,018 confirmed dengue cases. Although dengue has been a notifiable disease since 1996, regional comparisons suggest that reported cases does not reflect the full impact of the disease. A selective surveillance system, challenges in diagnosis, and paucity of economic studies all restrict policy makers' ability to understand the true burden of dengue and improve its control. We report findings from four-part national study to analyze the economic cost of dengue illness in India. Part one is estimating the cost of dengue hospitalizations to the Indian health system based on a retrospective study from 10 medical colleges across five regions of India. A randomly selected sample of 1448 medical records of hospitalized

dengue patients, between the years 2006 through 2011, were abstracted using a standardized questionnaire, obtaining length of hospital stay and other variables. Part two, a prospective study of 244 randomly selected ambulatory suspected dengue cases interviewed between October 2012 and March 2013, provides information on ambulatory services. Part three combines financial information, bed capacity, occupancy, and aggregate ambulatory visits in a macro costing approach to estimate the cost of a hospitalized bed day equivalent and an ambulatory visit. Part four, based on a case study in Madurai, a Delphi panel, and national surveillance data derives an adjustment factor for reported cases to project the true number of dengue cases nationally. Preliminary results suggest an average inpatient case cost $245.49 based on an average 7.09 day hospital stay and a cost per bed-day equivalent of $29.26 plus $38.04 for 4.4 ambulatory visits. An average ambulatory case cost $38.04 based on 1.6 visits to hospital outpatient department and 2.9 visits to other outpatient providers. Our expansion factor of 47.1 suggests that India experienced on average 943,000 confirmed dengue cases annually from 2006-12. Of these cases 282,900 (30%) were treated in the ambulatory setting with an average annual cost of $11 million, and 660,100 (70%) were hospitalized with an average annual cost of $162 million. The total cost for all confirmed dengue cases per year was $173 million. These preliminary data indicate that the economic burden of dengue in India is substantial and that control measures merit serious consideration.

1095

PATHWAY TO GROWTH FALTERING: COMPARISON OF GROWTH MODELING TECHNIQUES

Stephanie A. Richard[1], Benjamin J. McCormick[1], Mark Miller[1], Laura E. Caulfield[2], William Checkley[2], On behalf of the MAL-ED Network

[1]Fogarty International Center, National Institutes of Health, Bethesda, MD, United States, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Growth is a common metric of the health status of a population, as it is relatively easy to measure and is associated with both short- and long-term health outcomes. Weight loss (or weight faltering) is known to be associated with acute insults (dietary insufficiency, infectious disease) whereas linear growth faltering is associated with cumulative, long-term burden of dietary insufficiency and infectious diseases. Wasting (weight-for-length Z-score <-2) and stunting (length-for-age Z-score <-2) have been associated with increased risk of mortality during childhood, and stunting has also been associated with lower educational attainment and economic productivity, as well as greater risk of chronic illness during adulthood. We will compare and contrast various methods to evaluate growth and growth faltering using data collected in eight countries using harmonized protocols as part of the MAL-ED cohort study. We will consider different approaches for looking at growth, including longitudinal models, splines, and continuous Z-scores, among others, and discuss the flexibility, interpretation, and merits of each approach for specific research questions.

1096

EFFECT OF ARTESUNATE TREATMENT ON *PLASMODIUM FALCIPARUM IN VIVO* GENOMIC EXPRESSION

Aminatou Kone[1], Antoine Dara[1], Amadou Niangaly[1], Indranil Sinha[2], David Brodin[2], Bakari Fofana[1], Souleymane Dama[1], Demba Dembele[1], Bakari Sidibe[1], Mahamadou Aly Thera[1], Isiaka Sagara[1], Karin Dalman Wright[2], Anders Bjorkman[2], Joze Pedro Gil[2], Ogobara Doumbo[1], Abdoulaye Djimde[1]

[1]Malaria Research and Training Center, Bamako, Mali, [2]Karolinska Institutet, Stockholm, Sweden

Artemisinin-based combination therapies are the main treatment for malaria in endemic countries. Both the mechanism of action and the mechanism of resistance to artemisinins are poorly understood. Transcriptomic studies can help in improving our understanding of these processes. During a prospective study of the efficacy of artesunate in monotherapy in children aged 1-10 years and presenting with uncomplicated *falciparum* malaria in Bougoula-Hameau, Mali, venous blood was collected before treatment (H0) and one (H1), two (H2) and three hours (H3) after treatment. RNA was extracted from these respective blood samples and used for microarray experiments with *Plasmodium/Anopheles* chips using the AffymetrixR platform. A total of 24 samples from 6 patients were included in the final analysis after quality control . For each patient differential gene expression was measured and expression levels at baseline (H0) was compared to levels at H1, H2 and H3 after treatment. Heat shock proteine 70, several ubiquitines and a number of AP2 domain-containing genes were significantly up-regulated in the immediate hours following artesunate treatment. We show that genes involved in cell cycle regulation, DNA damage repairs and response to heat shock were up-regulated in *P. falciparum* from patients treated with artesunate. Our data support a role for these genes in the *in vivo* response of *P. falciparum* to artesunate.

1097

STUDY OF VAR GENES EXPRESSION IN SEVERE MALARIA IN IMPORTED MALARIA CASES

Nicolas Argy[1], Gwladys Bertin[2], Valérie Briand[2], Veronique Hubert[1], Philippe Deloron[2], Sandrine houzé[1]

[1]Bichat-Claude Bernard Hospital, French Malaria National Reference Center, Paris, France, [2]UMR 216, Paris Descartes University, Paris, France

In the physiopathology of severe malaria (SM) caused by *Plasmodium falciparum*, the phenomenon of cytoadherence between parasitized red blood cells (PRBC) and cell receptors of the host plays a fundamental role. This interaction is mediated by PfEMP1, parasitic protein expressed on the surface of PRBC. Var genes encoding theses proteins are classified into several groups: A, B, C, B/A, B/C and var 1-4. Studies in the field demonstrated a relation between the var gene expression and the clinical presentation of the malaria case according to the age of the patients. The aim of this work was to study the expression of these var genes for the first time in imported malaria according to the ethnicity of patient (Caucasian, African immigrants and children of African immigrants born in France), the clinical presentation (SM vs. uncomplicated malaria (UM)) and the immune status of the host. A prospective study was done on imported malaria cases observed in metropolitan France and reported to the malaria national reference center in France. Epidemiological, clinical and biological data was collected for all included cases of SM and UM caused by *P. falciparum*. From each blood sample before treatment, DNA and RNA were extracted respectively for multiplication of infection (MOI) determination and for RT PCR to study var gene expression. Plasma was also collected for immunologic tests. Eighty malaria imported cases (31 SM and 49 UM) from 19 West African countries and with different ethnicities (14 Caucasian, 57 African immigrants and 9 children of African immigrants born in France) were included in the study. var gene expression studies by RT PCR revealed that var group A and B were more expressed in SM than in UM. This expression was also influenced by the ethnicity of the host: var group A was predominantly expressed in non immune patients (Caucasian and children of African immigrants born in France) during SM whereas var group B was predominant in African immigrants. These results were consistent with prior published data in studies of non immun children in endemic areas (Rottmann et al., Infect Immun, 2006). The immune status of the host seems to influence which var gene group is expressed and the occurrence of SM but this observation has to be further investigated.

1098

ELEVATED PHENYLALANINE LEVELS ARE ASSOCIATED WITH OXIDIZED BIOPTERIN IN TANZANIAN CHILDREN WITH MALARIA: A POSSIBLE BIOMARKER OF OXIDATIVE STRESS

Matthew P. Rubach[1], Jackson Mukemba[2], Salvatore Florence[2], Bernard John[2], Bert K. Lopansri[1], Bert K. Lopansri[3], Tsin W. Yeo[4], J. Brice Weinberg[1], Nicholas M. Anstey[4], Donald L. Granger[5], Esther D. Mwaikambo[2]

[1]Duke University and Veterans Affairs Medical Centers, Durham, NC, United States, [2]Hubert Kairuki Memorial University, Dar es Salaam, United Republic of Tanzania, [3]Intermountain Healthcare, Salt Lake City, UT, United States, [4]Menzies School for Health Research and Charles Darwin University, Darwin, Australia, [5]University of Utah School of Medicine and Veterans Affairs Medical Center, Salt Lake City, UT, United States

We previously reported hyperphenylalaninemia (HPA) in children with malaria. The mechanism underlying this metabolic anomaly is unknown. In healthy states, plasma phenylalanine (Phe) concentration is tightly regulated by substrate-level activation/inhibition of hepatocyte phenylalanine hydroxylase (PAH), the enzyme which mono-oxygenates Phe to tyrosine. A biopterin species, tetrahydrobiopterin (BH_4), is a required cofactor for PAH catalysis. PAH is activated as Phe levels rise and is inhibited by the product of BH_4 oxidation, dihyrobiopterin (BH_2). Under normal conditions, biopterin is maintained largely reduced as BH_4. We hypothesized that HPA in malaria reflects oxidative stress placed on the biopterin redox pathway and thus HPA would correlate with elevated BH_2 levels. We addressed this hypothesis by quantifying the oxidized (BH_2 and biopterin [B]) and reduced (BH_4) biopterin species in urine, the most accurate method available for measuring total body metabolism of these compounds. Children 6 months to 6 years of age presenting at two district hospitals in Dar es Salaam with cerebral malaria (CM, n=53) and uncomplicated malaria (UM, n=67) were prospectively enrolled. Similar aged, healthy children (HC, n=116) and children presenting with non-malarial CNS conditions (NMC, n=53) were prospectively enrolled as controls. Plasma Phe was measured by ion exchange chromatography. Biopterins were measured by HPLC with fluorescence and electrochemical detection on urine collected into redox stabilizer + chelator to prevent *ex vivo* oxidation. In HC 3/110 had Phe levels above the upper limit of the normal range (82uM), in contrast to 22/50 NMC, 46/63 UM and 38/50 CM cases. Phe levels varied significantly across the 4 groups (p=0.0001, Kruskal-Wallis). Of the 3 species of biopterins (B, BH_2, BH_4) and the reduced:oxidized ratio [BH_4:BH_2+B], plasma Phe correlated best with BH_2 concentration (r=0.53, p<0.0001, Spearman correlation). These data suggest that HPA in UM and CM may result from increased inhibition of PAH due to elevated BH_2 levels. Elevated BH_2 may arise when sufficient reducing power (NADH/NADPH) is unavailable in hepatocytes for recycling BH_2 to BH_4. An intracellular environment rich in oxidants might produce such conditions. Biopterin redox status may be uniquely sensitive to oxidation and thus HPA, while not specific to malaria, could serve as an easily measurable surrogate for oxidative stress.

1099

ERYTHROCYTIC CYCLE DURATION AND SYNCHRONICITY OF DIFFERENT *PLASMODIUM FALCIPARUM* CLONAL LINES

Nestor A. Agbayani, Lindsey B. Turnbull, Katrina A. Button-Simons, Michael T. Ferdig

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

Close correlation between the synchronous 48-hour cell cycle of *Plasmodium falciparum* and the human circadian rhythm suggests a link to virulence that depends on mechanisms of cycle duration and synchronicity of the asexual intraerythrocytic forms of the malaria parasite. The genetic determinants and molecular mechanisms regulating *P. falciparum* growth and development are not well characterized. Therefore, analysis of the relationship between cycle duration, synchronicity, and growth of the malaria parasites during the intraerythrocytic cycle will provide a deeper understanding of the underlying genetic mechanisms involved. We measured in triplicate the *in vitro* growth and synchronicity of three parasite strains every 8 hours over several cell cycles to build a precise compendium of these important phenotypes and their divergence across genotypes. Our data demonstrate that rate of synchronicity loss is a distinct feature of individual parasite clones: HB3 and 3D7 had similar cycle lengths and loss of synchronicity rates, while Dd2 had a significantly shorter cycle duration and lost synchrony more quickly. Our data suggest that the cultured parasite, without any cues from the host, has some control over its synchronous state. An Agent Based Model was generated utilizing these and previously published data to model intraerythrocytic parasite dynamics over time. These analyses suggest a relationship between cycle length and synchronicity that can be used to predict parasite growth, invasion efficiency and other phenotypes that influence virulence and interaction with the human host.

1100

FUNDAMENTAL INSIGHTS TOWARDS IN VITRO ANALYSES OF *PLASMODIUM*-HEPATOCYTE INTERACTIONS: PROTEOMIC COMPARISONS OF SUSCEPTIBLE AND REFRACTORY CELL LINES

Jonas G. King[1], Dingyin Tao[1], Ceereena Ubaida Mohien[2], David Graham[2], Philipp Jost[3], Justin Boddey[4], Rhoel D. Dinglasan[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Department of Molecular and Comparative Pathobiology, Johns Hopkins School of Medicine, Baltimore, MD, United States, [3]Medizinische Klinik, Klinikum rechts der Isar, München, Germany, [4]Division of Infection and Immunity, The Walter and Eliza Hall Institute, Parkville, Australia

Our long-term aim is to reveal the full complement of proteins deployed by the exo-erythrocytic (EEF) liver-stages of human *Plasmodium* species and to understand how these parasites interact with host hepatocytes. We are especially interested in factors involved in cellular invasion, parasitophorous vacuole formation, and modulation of host cell death, as the ability to prevent cell death is likely a significant selective pressure on the parasite. Here, we address basic questions of cellular biology regarding a human cell line, HC-04, which is capable of supporting *P. falciparum* and *P. vivax* EEF development at low intensities. Using shotgun proteomics, and focused transcriptomics and immunohistochemistry, we have begun answering essential questions regarding (i) the optimization of *in vitro P. falciparum* invasion and development, (ii) HC-04 transcriptomic and proteomic profiles in the context of apoptosis vs. proliferation, and (**iii**) the glycoproteomic analysis of HC-04 relative to human hepatocyte lines that do not support the development of human malaria EEF stages. Comparative analyses or our results identify putative players in *Plasmodium*-hepatocyte interactions. This effort also lays a framework for more directed experiments, including ongoing analyses involving the effect of *Plasmodium* proteins ectopically expressed in human hepatocytes.

1101

MULTICLONAL ACTIVATION OF HYPNOZOITES IN RETURN TRAVELERS SUFFERING *PLASMODIUM VIVAX* RELAPSE

Jessica T. Lin[1], Laurel Kartchner[1], Dylan R. Pillai[2], Nicholas Hathaway[3], Jeffrey A. Bailey[3], Jonathan J. Juliano[1]

[1]University of North Carolina, Chapel Hill, NC, United States, [2]University of Calgary, Calgary, AB, Canada, [3]University of Massachusetts, Worcester, MA, United States

In endemic areas, *Plasmodium vivax* relapses are difficult to distinguish from new infections. Genotyping of travelers who suffer relapse after returning to a malaria-free area can be used to shed light on the nature of hypnozoite activation and relapse. To examine multiplicity of infection (MOI) in relapsing *P .vivax* infections, we applied three different genotyping methods - microsatellites, heteroduplex tracking assay (HTA), and Ion Torrent amplicon deep sequencing - to recurrent *vivax parasitemia*s in two travelers who returned to Canada after acquiring

vivax infection overseas, one in Pakistan and one in Honduras. In both travelers, multiclonal relapsing parasite populations were found. While the HTA, which assessed MOI based on sequence variation at the merozoite surface protein 1 (*pvmsp1*) gene, revealed 2-3 parasite variants at each of the 5 relapse episodes, microsatellite analysis only revealed multiclonal infection in 3/5 relapse episodes as measured by the maximum number of alleles detected at 4 neutral microsatellites markers. In one traveler, the HTA revealed a unique hypnozoite variant at second relapse that was not detected at first relapse. Aside from this, variants at consecutive relapses were genetically identical. These results highlight the propensity for multiple hypnozoite clones to activate simultaneously to cause relapse. Results from Ion Torrent sequencing of *pvmsp1* will be used to delve deeper into the degree of polyclonality found at relapse. In particular, we are interested in whether parasite variants in the same person change in frequency with consecutive relapses, reflecting perhaps different propensities for hypnozoite dormancy, reactivation, or immune evasion.

1102

PHENOTYPIC CHARACTERIZATION OF PERUVIAN *PLASMODIUM FALCIPARUM* HRP2 NEGATIVE ISOLATES: A PRELIMINARY STUDY

Elizabeth Villasis[1], Ronald Bautista[1], Jorge Bendezu[1], Katherine Torres[1], Sara Lustigman[2], Joseph Vinetz[3], Dionicia Gamboa[1]

[1]Laboratorio de Malaria, Laboratorio Investigacion para el Desarrollo, Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Molecular Parasitology, Lindsley F. Kimball Research Institute, New York Blood Center, New York, NY, United States, [3]Division of Infectious Diseases, Department of Medicine University of California San Diego, San Diego, CA, United States

In 2010, *Plasmodium falciparum* wild isolates lacking HRP2 protein were found in Peru. Since then, several reports had published the distribution of these parasites in different countries and their implication in the performance of malaria RDTs. Although the function of HRP2 protein is unknown, this unique featured lead us to the study of the phenotype of these isolates in terms of parasite multiplication rates, invasion profile and characteristics associated with virulence. Two groups of isolates were evaluated and adapted to culture: Four HRP2 negative isolates and four HRP2 positive isolates. All isolates were evaluated for parasite multiplication rate (PMR), invasion profile, rosseting formation, adhesion to CSA protein and HUVEC cell line. *P. falciparum* strains, HB3, Dd2, 3D7, FCR3, ITG and CS2 were used as controls in the correspondent assays. PRM values were similar between HRP2 negative isolates (4.1± 2.5) and HRP2 positive isolates (5.0 ± 2.4). No differences were found for the invasion profile between both groups. None of these parasites were able to form rosettes. Only one HRP2 positive isolate was able to interact with CSA protein and only two HRP2 negative isolate were able to interact with HUVEC cell line. This is the first report that characterizes the phenotype *P. falciparum* HRP2 negative isolates and compares them with the phenotype of HRP2 positive isolates and laboratory strains. This preliminary study shows that the phenotype features of HRP2 negative isolates are very similar to the HRP2 positive isolates in Peru. Since HRP2 negative parasites are widely distributed, it is important to continue the study of the phenotypic characteristics of these parasites and whether or not their impact in the prevention, control or eradication measures against malaria that are now taking place around the world.

1103

NEAR-INFRARED FLUORESCENT IMAGING: A NOVEL TECHNIQUE TO ASSESS BRAIN PATHOLOGY AND SEVERITY IN EXPERIMENTAL CEREBRAL MALARIA

Fernando Pereira Bruno[1], Henry J. Shikani[1], Minxian Dai[1], Brandi D. Freeman[1], Jasmin Jasmin[2], Herbert B. Tanowitz[1], Louis M. Weiss[1], David C. Spray[1], Mahalia S. Desruisseaux[1]

[1]Department of Pathology, Albert Einstein College of Medicine, Bronx, NY, United States, [2]Departamento de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Cerebral malaria (CM) is a severe neurological complication of *Plasmodium falciparum* infection. It is characterized, in part, by severe cerebrovascular damage and inflammation, and deleterious effects on blood brain barrier (BBB) integrity. Matrix metalloproteinase (MMP) enzyme activity has been shown to strongly correlate with inflammation and has been studied in the context of several CNS diseases, including CM. We developed a novel imaging technique which allows *in vivo* investigations of brain pathology in our experimental model of CM (ECM). In the present study, we examined MMP activity and BBB structure and function during the course of ECM in mice infected with *Plasmodium berghei* ANKA (PbA), using near-infrared fluorescent (NIRF) imaging and compared our findings to mice with non-neurotropic severe malarial disease (*Plasmodium berghei* NK65) and uninfected mice. BBB disruption, was confirmed via quantification of Evans Blue extravasation, and MMP abundance and activity was verified by immunoblots and immunohistochemistry. NIRF imaging demonstrated significantly higher MMP activation in several brain regions in PbA mice, including in the brain stem, cerebellum, hypothalamus and cortex. This activation significantly progressed over time, correlating with increasing severity of disease, abnormalities in histological findings and the temporal onset of behavioral dysfunctions in ECM. In addition, *in vivo* imaging of the BBB demonstrated a progressive increase of BBB leakage in ECM mice, concomitant with the rise in MMP levels. Evans Blue quantification of *ex vivo* mouse brains corroborated our NIRF observations. Our data demonstrate that NIRF provides a sensitive and reliable approach to monitor the progression of ECM by allowing unprecedented direct visualization of neuroinflammation and BBB disruption, in a non-invasive manner, during the course of disease. This novel imaging technique may prove to be valuable in monitoring treatment of CM.

1104

HETEROLOGOUS *PLASMODIUM VIVAX* RECURRENCES DUE TO MINORITY VARIANT EXPANSION

Jessica T. Lin[1], Chanthap Lon[2], Nicholas Hathaway[3], Oksana Kharabora[1], Jeffrey A. Bailey[3], Charlotte Lanteri[2], Panita Gosi[2], David Saunders[2], Jonathan J. Juliano[1]

[1]University of North Carolina, Chapel Hill, NC, United States, [2]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [3]University of Massachusetts, Worcester, MA, United States

Malaria recurrence following *Plasmodium vivax* infection can be due to relapse, reinfection, or recrudescence. Molecular genotyping studies which have compared genotypes of initial and recurrent *vivax* infections often demonstrate heterologous recurrence (i.e. different genotypes than those seen initially), even in the setting of known relapse. We investigated whether heterologous relapse can be due to expansion of minority variants (variants present at less than 20% of the parasite population) originally present in the initial infection but undetected by standard genotyping methods. Using the Ion Torrent sequencing platform, we deep sequenced 133bp of the polymorphic *P. vivax* merozoite surface protein-1 gene in isolates collected from 69 Cambodians with *P. vivax*. In this cohort, 22 persons developed one or more recurrences (range: 1-3) a median of 68 days following treatment with dihydroartemisinin-piperaquine without primaquine. All samples were amplified and sequenced in duplicate, with mean 3,460x coverage. Sequences were defined as unique variants if they were present in both samples at ≥0.5% frequency and not determined to

be chimeras. We identified 50 unique *pvmsp1* haplotypes in the cohort. The mean multiplicity of infection was 3.7 (range 1-13 variants per person), with initial and recurrent episodes equally polyclonal. One-third of recurrences (9/29) had the same dominant or co-dominant haplotype/s at recurrence as seen in the preceding episode. Of the remaining 20 heterologous recurrences, nearly half (8/20) could be attributed to expansion of a minority variant seen in the initial infection. Reappearance, or outgrowth, of an originally minor population might occur randomly in a stochastic fashion, or it might occur if certain parasite variants are predisposed to hypnozoite formation and relapse. Variant "A", was responsible for half of the heterologous recurrences arising from minority variant expansion. However, it was also the most common variant overall, existing as the dominant variant in 9% of all initial infections and a minority variant 25% of the time.

1105

CLINICAL PROFILE OF MALARIA IN COLOMBIA AND THEIR ASSOCIATION WITH HOST IMMUNOLOGICAL STATUS

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

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

Human infections by *Plasmodium* species have a broad clinical spectrum ranging from asymptomatic to severe malaria and complicated cases leading to death. The complex host-parasite interactions as well as geographic and social factors play a role in malaria pathogenesis. Although Colombia has one of the highest malaria incidences in the continent, few studies have documented the clinical profile and outcome of malaria infections in the country characterized by unstable transmission. One of the priorities of our NIH-ICEMR program is to generate a detail understanding of the clinical features of malaria infections in regions with different epidemiological conditions. We enrolled a total 1,600 individuals 1-80 y/age, attending outpatient clinics in Tumaco, Quibdó and Tierralta, three of the field sites of CLAIM, characterized by different epidemiological conditions, where both *P. falciparum* and *P.vivax* are prevalent with diverse transmission intensities. Participants provided a written informed consent/ascent and were subjected to clinical evaluation and laboratory tests (urine and blood) including malaria serology. Malaria cases were more frequent among young adults and housewives, students and fishermen. The majority of enrolled individuals (93%) presented with uncomplicated malaria. Few cases (n=3) of severe anemia (Hb <7g/dL) and severe thrombocytopenia (<20,000 ptt/µL) (n= 2) were observed, all caused by *P.vivax*. Mild anemia (Hb<11g/dL) was observed in 20% of cases and 48% displayed mild thrombocytopenia (<150,000/µL). Although, 11 individual presented high ALT levels (>120 U/L), no correlation with clinical manifestation was observed. High creatinine levels (>1.5 mg/dL) without any further renal manifestation were observed in 5 patients, three of them with *P.falciparum* malaria. Antibodies against *P. vivax* and *P. falciparum* blood stages were found in 60% and 27% of the studied population respectively, at low IFAT titers (≤1:80). The prevalence of IgG antibody reactivity to both PvCSP and PvMSP-1 was ~50%, most of them with titers ≤1:400. The high prevalence of uncomplicated malaria together with the low antibody titers appear to be associated with the low transmission intensity and may indirectly indicate an early diagnose and effective treatment and reflects the low mortality (~20 cases/2012) recorded in Colombia during the last few years.

1106

EVIDENCE FOR THE O-LINKED-β-*N*-ACETYLGLUCOSAMINE MODIFICATION IN *PLASMODIUM*

Rebecca Pastrana-Mena[1], Derrick Mathias[1], Dingyin Tao[1], David R. Colquhoun[2], Rhoel R. Dinglasan[1]

[1]Johns Hopkins University School of Public Health, Baltimore, MD, United States, [2]Johns Hopkins School of Medicine, Baltimore, MD, United States

The O-linked-β-*N*-acetylglucosamine (O-GlcNAc) is a dynamic post-translational modification, occurring in nuclear and cytosolic compartments, that has been shown to be an important mediator of stress response, intracellular signaling and cell survival among others. While O-GlcNAc has been well studied in Metazoans little is known about this modification and its role in the cellular biology of Protozoans, including apicomplexan parasites in the genus *Plasmodium*. The causative agents of malaria, *Plasmodium spp.*, undergo a very complex life cycle which includes development of the parasite within the host's red blood cell (RBC) and subsequent extracellular development of the parasite inside the mosquito vector. Both environments provide numerous stressors for parasite development, and to survive, it must rely on a very efficient defense response. If the O-GlcNAc modification is indeed present in *Plasmodium*, its role as a mediator of stress in other organisms suggests the potential for a similar role in the parasite. A close examination into the presence and possible roles of O-GlcNAc in malaria biology has not been adequately and properly performed. To date the enzymes responsible for the addition and removal of this modification remain unknown in *Plasmodium*, and previous studies in asexual stages have prevented the unequivocal demonstration of this modification due to the presence of contaminating host RBC O-GlcNAc modified proteins. We performed Western blot analysis using specific O-GlcNAc antibodies and proteins purified from ookinetes and sporozoites, both extracellular stages of the parasite, and confirm the presence of O-GlcNAc-modified proteins in *P. berghei* and *P. falciparum*. Addition of the modification onto parasite proteins was increased after drug treatment suggesting a role in stress response as hypothesized. Immunofluorescence assays of ookinete and sporozoite stages showed the presence of this modification in the cytosol of the parasite. Additionally, we have identified using mass spectrometry a subset of proteins with predicted O-GlcNAc modification sites. In an effort to identify the parasite OGT and OGA we used a bioinformatic approach to identify two candidate genes for OGT and one candidate gene for OGA. To test whether we identified putative homologs of OGT and OGA, we generated knockouts of the candidate *loci* in *P. berghei* and evaluated their role in O-GlcNAc modification.

1107

INVESTIGATING MECHANISMS OF PYRETHROID RESISTANCE IN FIELD POPULATIONS OF *ANOPHELES FUNESTUS* IN SOUTHERN AFRICA

Kayla G. Barnes, Michael Coleman, Jacob Riveron Miranda, Janet Hemingway, Charles S. Wondji

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Mechanisms underlying insecticide resistance of *Anopheles funestus*, a major malaria vector, remain uncharacterized. In this study, WHO bioassays carried out on An.*funestus* from Mozambique, Malawi, and Zambia indicate that pyrethroid and carbamate resistance previously detected in southern Mozambique has spread northward while full susceptibility remains for organophosphates. Piperonyl butoxide (PBO) synergist assays indicated that a metabolic resistance mediated by cytochrome P450s is the likely cause of pyrethroid resistance. Microarray analysis identified three main P450 genes (CYP6P9a, CYP6P9b, and CYP6M7) which varied in expression levels between countries. Lower expression levels were observed for the duplicated CYP6P9a and CYP6P9b in central and northern Malawi and Zambia while CYP6M7 expression was higher in these locations. The up-regulation of these genes was validated by qRT-PCR. Using transgenic expression of these proteins we were able

to functionally metabolize both type 1 and type II pyrethroids but not carbamate. Other genes potentially involved in resistance are short chain dehydrogenases, ABC transporters (ABCB7), and other P450s such as CYP6AA4 and CYP9J11. To further investigate the genetic differences between these populations (N=10) we analyzed 48 field isolates from ten locations using 17 microsatellites spanning the genome. We observed a significant difference between populations at the geographical and temporal level (pairwise Fst score p≤0.05). These results parallel our microarray data and we are currently sequencing the rp1(6P9a/b) and rp2(6M7) Quantitative Trait *Loci* to pinpoint genetic differences the extent of selection in these important P450s and show how mechanisms of resistance vary between populations. Further ongoing analyses will help to establish a comprehensive picture of mechanisms controlling pyrethroid resistance in field populations of this important malaria vector.

1108

MITOCHONDRIAL DNA VARIATION IN *ANOPHELES PSEUDOPUNCTIPENNIS THEOBALD* (*DIPTERA: CULICIDAE*) FROM COLOMBIA, SOUTH AMERICA

Giovan F. Gomez[1], Ranulfo González[2], Natali Avendaño[1], Margarita M. Correa[1]

[1]Universidad de Antioquia, Medellin, Colombia, [2]Universidad del Valle, Cali, Colombia

Anopheles pseudopunctipennis plays an important role as malaria vector in Central and South America. In Colombia, it is a suspected local vector and nothing is known about its population structure. This study used a 624 bp fragment of the mitochondrial Cytochrome Oxidase I gene to explore genetic variation in *An. pseudopunctipennis* natural populations collected in six localities of three Departments: Antioquia-A (*n*=21), Cordoba-C (*n*=23) and Valle del Cauca-V (*n*=33). Forty-four haplotypes and a high haplotype diversity for all populations (*Hd*=0.73810-0.97538) were detected. The nucleotide diversity was higher in C (*Pi*: 0.01638), followed by A (*Pi*: 0.00797) and V populations (*Pi*: 0.00607). Also, the highest average number of nucleotide differences (*K*= 10.20553) was detected for C. Significant negative values in the Tajima's D and Fu's *Fs* tests for the V population indicated possible population expansion or natural selection. While, C and A populations showed positive and no significant results. The *Fst* pairwise comparison between populations showed only significant differences ($p<0.05$) between Valle del Cauca and each of the others. The highest level of genetic differentiation (*Fst*=0.49941, N_M=0.50) was detected between the Cordoba and Valle del Cauca populations. These results indicate that the Cordoba and Antioquia *An. pseudopunctipennis* populations are genetically similar, while a different gene pool is present in Valle del Cauca with little or no current gene flow. This study shows genetic structure of *An. pseudopunctipennis* along its distribution range and constitute a first approach to the genetic study of this species in Colombia.

1109

HUMAN ENCROACHMENT ON FORESTS MAY INCREASE EXPOSURE TO NEW MOSQUITO SPECIES AND PATHOGENS EVIDENCE FROM ZIKA FOREST, UGANDA

Martha A. Kaddumukasa[1], Jonathan K. Kayondo[2], Anne M. Akol[1], Daniel Masiga[3], Julius J. Lutwama[2], Charles Masembe[1]

[1]Makerere University, Kampala, Uganda, [2]Uganda Virus Research Institute, Entebbe, Uganda, [3]International Centre for Insect Physiology and Ecology (ICIPE) African Insect Science for Food and Health, Nairobi, Kenya

The steady increase in contact between humans and wildlife is brought about by human encroachment, destruction of natural forests and environmental changes. Mosquitoes get exposed to new hosts and pathogens; creating possibilities for new disease patterns. Therefore identification of blood meal sources is important to determine the interaction between hosts and vectors. In this work, engorged mosquitoes were collected in Zika forest (Uganda) for a period of 12 months, and abdominal contents sequenced for c*ytochrome oxidase subunit I* and *cytochrome b*. The sequences were subsequently blast searched in Genbank and the analyses revealed the presence of mammalian (86%), avian (13%) and amphibian-derived (1%) hosts. The first record of human origin blood-meals from *Uranotaenia* species in Zika Forest was shown. Earlier studies showed these species to feed exclusively on reptiles, amphibians, birds or domestic mammals. Taking of mammalian origin blood-meals puts the human and entire animal community at risk because of the possibility of exposure to new pathogens. Significant differences between host species were observed (Kruskal Wallis test, χ^2 = 19.118, df = 5, p = 0.018) suggesting a wide range of host exposure. This could possibly create new disease patterns. Several mosquitoes may be considered potential bridge vectors for a number of arboviruses from the composition of their blood-meals. These results highlight the public health significance of taking measures to avoid encroachment of forests and reserves for diseases prevention and control.

1110

REVERSE-GENETIC AND PHARMACOLOGIC STRATEGIES FOR PROBING MOSQUITO INWARD RECTIFIER POTASSIUM CHANNELS AS INSECTICIDE TARGETS

Rene Raphemot[1], Emily Days[1], Tania Y. Estevez-Lao[1], Daniel Swale[1], Matthew F. Rouhier[2], Julián F. Hillyer[1], C. David Weaver[1], Corey R. Hopkins[1], Klaus W. Beyenbach[3], Peter M. Piermarini[2], Jerod S. Denton[1]

[1]Vanderbilt University Medical Center, Nashville, TN, United States, [2]The Ohio State University, Wooster, OH, United States, [3]Cornell University, Ithaca, NY, United States

Diseases such as malaria and dengue fever are transmitted through the bite of infected mosquitoes during blood feeding. Insecticides remain the most effective means to kill mosquitoes and prevent the transmission of these devastating diseases; however, the continued emergence of insecticide resistance in mosquito populations has reduced their efficacy. Thus, the development of new mosquitocides is critically needed to slow the spread of mosquito-borne diseases. Inward rectifier potassium (Kir) channels play key physiological roles in epithelial, nerve, and muscle cells in mammals. However, their functions and potential as insecticide targets in mosquitoes have remained largely unexplored. We recently showed that a small-molecule inhibitor of *Aedes aegypti* Kir1 elicits 'renal failure' in mosquitoes and leads to death. Since then, a high-throughput screen of more than 40,000 compounds led to the discovery of several additional small-molecule inhibitors of *Aedes* Kir1, and they are now being evaluated as potential insecticides. Furthermore, to begin elucidating the functions of Kir1 in *Anopheles gambiae*, we used RT-PCR analysis to reveal that Kir1 is expressed in several tissues, including the ovaries, suggesting that Kir1 may contribute to mosquito reproduction. To test this idea, RNA interference was employed to knock down the expression of Kir1. Pilot studies suggest that knock-down of Kir1 decreases mosquito fecundity. The use of these novel molecular and pharmacological tools will be useful in further evaluating the potential of Kir1 as an insecticide target in disease-carrying mosquitoes.

1111

CYTOGENETIC ANALYSIS REVEALED INVERSION POLYMORPHISM IN A MAJOR MALARIA VECTOR IN CENTRAL AFRICA, *ANOPHELES MOUCHETI*

Maria V. Sharakhova[1], Christophe Antonio-Nkondjio[2], Ai Xia[1], Cyrille Ndo[2], Cyrille Ndo[2], Parfait Awono-Ambene[2], Frederic Simard[3], Igor V. Sharakhov[1]

[1]Virginia Tech, Blacksburg, VA, United States, [2]Malaria Research Laboratory, OCEAC, Yaounde, Cameroon, [3]Institut de Recherche pour le Développement, IRD, Montpellier, France

Anopheles moucheti is a primary vector of malaria in forested areas of Central Africa. A recent study conducted in Gabon suggests that

this species is a major candidate vector for the possible transfer of *Plasmodium* parasite from apes to human. Epidemiologically important adaptations and behaviors of major malaria vector in Africa, *An. gambiae* are associated with the polymorphism of paracentric chromosomal inversions. However, population genetic and cytogenetic studies on *An. moucheti* have not been performed. The current study is the first attempt to characterize polytene chromosomes in *An. moucheti* females collected in three locations in Cameroon. We demonstrated that ovarian nurse cells contain readable polytene chromosomes, which are suitable for all standard cytogenetic applications. The homology between 2R chromosomal arms of *An. moucheti* and *An. gambiae* was established by fluorescent *in situ* hybridization of six *An. gambiae* genic sequences. Positions of the probes on chromosomes of *An. moucheti* detected substantial gene order reshuffling between the two species. A population analysis revealed the presence of three highly polymorphic chromosomal inversions in *An. moucheti*. Two of the inversions are located on the 2R arm and one inversion is found on chromosome 3. The frequency of the heterozygotes in the populations for one or more inversions was 50%. The high level of the inversion polymorphism in *An. moucheti* may suggest a complex population structure and/or pattern of ecological adaptations and behaviors in this mosquito. Interestingly, populations of other major vectors, *An. gambiae, An. arabiensis, An. funestus,* and *An. nili* have significantly reduced inversion polymorphism in Central Africa. Our study lays a foundation for a more detailed characterization of inversion polymorphism in *An. moucheti* and will stimulate population genetic, taxonomic, and genomic studies of this neglected malaria vector.

1112

ROLE OF MIRNAS IN 'FINE-TUNING' THE *ANOPHELES GAMBIAE* IMMUNE RESPONSE TO *PLASMODIUM FALCIPARUM*

Nathan Dennison, George Dimopoulos

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

The transcriptional response of *Anopheles gambiae* to *Plasmodium* infection has been well documented, identifying a suite of immune genes involved in the host defense response. In recent years, post-transcriptional regulation of gene expression by microRNAs (miRNAs) has garnered increased attention. miRNAs are small non-coding RNAs responsible for post-transcriptional regulation in a sequence specific manner. miRNAs are transcribed by RNA Pol II to form pri-miRNAs, cleaved by Drosha to form pre-miRNAs and then by Dicer-1 into their mature form (21-25 nt). Argonaute-1 (Ago-1) then guides the mature miRNA to target mRNA 3' untranslated regions. We have demonstrated that RNAi targeting of *ago-1* and *dicer-1* cause a reduction in *P. falciparum* infection intensity, most notably when targeting *ago-1*. This indicates that preventing miRNAs from regulating target genes decreases survival of the parasite, suggesting a possible role for miRNAs in regulating the *Anopheles* anti-*Plasmodium* immune response and other mosquito factors that influence parasite infection To further investigate the role of miRNAs, we used a custom designed miRNA microarray profiling the differential abundance of 195 mosquito miRNAs between naïve and *P. falciparum* infected *A. gambiae* midguts when the parasite is invading this tissue. Currently there are 67 *A. gambiae* miRNAs annotated in mirBASE and here, we identified 32 midgut expressed miRNAs of which one was significantly upregulated by *P. falciparum* infection. Bioinformatics analysis suggests that this miRNA potentially post-transcriptionally regulates several innate immune genes, including the anti-*P. falciparum* IMD immune pathway factor *Imd* and effector *apl1B*. Therefore, antagomirs, chemically engineered oligonucleotides that silence endogenous miRNAs, are used *in vivo* to confirm the role of the miRNA in regulating the anti-parasitic immune response. Our data suggests that miRNAs are required to 'fine tune' the transcriptional response to *Plasmodium* invasion.

1113

POPULATION GENETIC STRUCTURE OF THE MALARIA VECTOR *ANOPHELES FUNESTUS* IN A RECENTLY RE-COLONIZED AREA OF SENEGAL RIVER BASIN AND HUMAN-INDUCED ENVIRONMENTAL CHANGES

Badara Samb[1], Ibrahima Dia[2], Lassana Konate[3], Diego Ayala[4], Didier Fontenille[4], Anna Cohuet[5]

[1]Laboratoire d'Ecologie Vectorielle et Parasitaire, Département de Biologie Animale, Université Cheikh Anta Diop de Dakar and Unité d'Entomologie Médicale, Institut Pasteur de Dakar, Dakar, Senegal, [2]Unité d'Entomologie médicale, Institut Pasteur de Dakar, Dakar, Senegal, [3]Laboratoire d'Ecologie Vectorielle et Parasitaire, Département de Biologie Animale, Université Cheikh Anta Diop de Dakar, Dakar, Senegal, [4]Institut de Recherche pour le Développement, Unité MIVEGEC, Montpellier, France, [5]Institut de Recherche pour le Développement, Unité MIVEGEC, Montpellier, France and Institut de Recherche en Sciences de la Sante, Bobo Dioulasso, Burkina Faso

Anopheles funestus is one of the major malaria vectors in tropical Africa. Because of several cycles of droughts events that have occurred during the 1970s, this species had disappeared from many parts of sahelian Africa, including the Senegal River basin. However, this zone has been re-colonized during the last decade by *An. funestus*, following the implementation of two dams on the Senegal River. Previous studies in that area revealed heterogeneity at the biological and chromosomal level among these recent populations. Here, we studied the genetic structure of the newly established mosquito populations using eleven microsatellite markers in four villages of the Senegal River basin and compared to another *An. funestus* population located in the sudanian domain. Our results presume Hardy Weinberg equilibrium in each *An. funestus* population, suggesting a situation of panmixia. Moreover, no signal from bottleneck or population expansion was detected across populations. The tests of genetic differentiation between sites revealed a slight but significant division into three distinct genetic entities. Genetic distance between populations from Senegal River basin and sudanian domain was correlated to geographical distance. In contrast, sub-division into the Senegal River basin was not correlated to geographic distance, rather to local adaptation. The high genetic diversity among populations from Senegal River basin coupled with no evidence of bottleneck and with a gene flow with southern population suggests that the re-colonization was likely carried out by a massive and repeated stepping-stone dispersion starting from the neighboring areas where *An. funestus* endured.

1114

GENETIC STRUCTURE OF *ANOPHELES (NYSSORHYNCHUS) GOELDII* (*DIPTERA: CULICIDAE*) IN THE BRAZILIAN AMAZON

Maysa T. Motoki[1], Marinete M. Povoa[2], Izis Sucupira[2], Jan E. Conn[1]

[1]Wadsworth Center, Griffin Laboratory, New York Department of Health, Albany, NY, United States, [2]Laboratorio de Pesquisas Basicas em Malaria, Instituto Evandro Chagas, Belem, Brazil

Specimens of the presumptive malaria vector *Anopheles goeldii* in the Nuneztovari Complex were investigated to test the hypothesis of population structure within the Brazilian Amazon Basin. Species identification was confirmed by sequencing for the mtDNA Folmer region and Bayesian Inference analysis was conducted with known samples of *An. goeldii*. Twelve microsatellite *loci* of specimens from seven locations in Brazil: Boa Visa and Iracema in northern Roraima State; Santana in Amapá State, north of the Amazon River; and Altamira, Itaituba, Mojú and Santarém south of the Amazon River in Pará State, were analyzed. Genetic variability was very high (RS = 20; HE = 0.805). There was deviation from HWE for 31.3% of single-locus tests due to heterozygote deficits, and linkage disequilibrium was not significant. Among populations, FST estimates ranged from 0.008-0.078 and were significantly different for 16/21 pairwise comparisons. Gene flow (Nm)

was >1 for all comparisons. There was no significant correlation between linearized FST and geographic distance. Effective population size (Ne) was moderately large for all populations regardless of model (linkage disequilibrium or heterozygote excess). STRUCTURE analysis detected three clusters; one was the most eastern locality, Mojú; the second was the northern localities, Boa Vista and Iracema, and the third was Altamira and Santana (Pará and Amapá States, respectively). Itaituba and Santarém in western Pará state consisted of admixed individuals belonging to all three clusters. Interestingly, Itaituba was also the site of multiple white and COI genotypes in the malaria vector An. *darlingi* (JEC and MMP, unpub.) and might be an Amazonian diversity hotspot. The factorial correspondence analysis indicated significant differences between Mojú and all other populations. Mojú is of particular interest because *An. goeldii* from this locality has been implicated as a local malaria vector for both *Plasmodium falciparum* and *P. vivax*. In summary, high diversity and significant population structure may contribute to phenotypic differentiation of *An. goeldii*, especially in Mojú, eastern Pará state.

1115

MULTIGENE PHYLOGENY OF MAJOR AFRICAN MALARIA VECTORS PLACES *ANOPHELES NILI* IN THE BASAL CLADE

Maryam Kamali[1], Christophe Antonio-Nkondjio[2], Cyrille Ndo[3], Zhijian Tu[1], Frederic Simard[4], Igor V. Sharakhov[1]

[1]Virginia Polytechnic Institute and State University, Blacksburg, VA, United States, [2]Malaria Research Laboratory, OCEAC, Yaounde, Uganda, [3]Faculty of Medicine and Pharmaceutical Sciences, University of Douala, Douala, Cameroon, [4]MIVEGEC (UMR IRD224-CNRS5290-UM1-UM2), Institut de Recherche pour le Développement (IRD), Montpellier, France

Anopheles gambiae, An. arabiensis, *An. funestus*, and *An. nili,* are among the major vectors of malaria in sub-Saharan Africa. *An. gambiae, An. arabiensis*, and *An. funestus* breed in temporal or permanent freshwater pools. However, *An. nili* breeds in slow-moving streams and large lotic rivers exposed to light. Phylogenetic analysis of vectors could be useful for understanding of association between evolutionary genomic changes and selective pressures from a malaria parasite on the immune systems of mosquitoes. In this study, we reconstructed a molecular phylogeny of major African malaria vectors and several outgroup species using 49 genes. These genes were evenly distributed throughout five chromosomal arms of *An. gambiae*. We identified orthologous sequences in the genomes of *An. nili*, *An. stephensi, Culex quinquefasciatus*, *Aedes aegypti*, and in the transcriptome of *An. funestus*. Phylogenetic trees were generated using neighbor-joining method from all individual genes and concatenated trees were obtained according to chromosomal arms in MEGA 5.05 program. Most of the trees obtained from individual genes were consistent, placing *An. nili* as the basal clade among the studied malaria mosquito species. Results were supported by high bootstrap values in concatenated phylogenetic trees generated separately for each chromosomal arm as well as individual trees. We conclude that the African *An. funestus* and Asian *An. stephensi* are the most closely related and most recently diversified lineages, while *An. nili* lineage split early from the other African *Anopheles* species and belongs to the basal clade. The multigene genome-wide molecular phylogenic approach could be useful in understanding the evolutionary relationships among malaria vectors.

1116

AN ANALYSIS OF *ANOPHELES'* OPSIN GENES IN RESPECT TO DIURNAL BEHAVIORAL VARIATION

Adam M. Jenkins[1], Alan S. Kopin[2], Marc A.T. Muskavitch[1]

[1]Boston College, Chestnut Hill, MA, United States, [2]Tufts Medical Center, Boston, MA, United States

Within the range of vector behaviors, we understand little about mosquito photopreference and the molecular mechanisms governing vision-dependent behavior in vector mosquitoes. Investigations of the influence of photopreference on mosquito behaviors such as endophagy/exophagy and endophily/exophily will enhance our ability to develop and deploy vector-targeted interventions and monitoring techniques. Previous laboratory-based analyses by our group have revealed that diurnal variation in photopreference and illumination intensity preference differ between *Anopheles gambiae* and *An. stephensi*. We are employing qRT-PCR to assess transcriptional expression patterns of long wavelength-, short wavelength-, and ultraviolet-sensing opsins (i.e., rhodopsin-class GPCRs) spanning two consecutive diurnal cycles, in *An. gambiae* and in *An. stephensi*. Using RNAseq data from *An. gambiae*, we are extending our analysis to the assessment of stage-specific transcriptional expression of genes implicated in phototransduction in first and third instar larvae, as well as in adult males and females. These analyses will provide insights into correlations between expression of phototransduction genes and diurnal photopreferences in *An. gambiae* and *An. stephensi*, and into the ensembles of phototransduction-associated genes expressed during larval and adult stages in *An. gambiae*. Our results will provide insights into molecular mechanisms involved in critical aspects of mosquito vision and the differential deployment of phototransduction machinery relevant to a number of vector behaviors.

1117

DYNAMICS OF KDR AND SPECIATION ISLAND INTROGRESSION BETWEEN *ANOPHELES GAMBIAE* MOLECULAR FORMS IN SOUTHERN MALI

Laura C. Norris[1], Yoosook Lee[1], Abdrahamane Fofana[2], Gregory Lanzaro[1]

[1]University of California Davis, Davis, CA, United States, [2]University of Mali, Bamako, Mali

Anopheles gambiae is the most important vector of *Plasmodium falciparum* malaria. Due to control failures such as emerging insecticide and drug resistance, genetically modified malaria-resistant mosquitoes are seen as a potential new method for malaria control. Malaria-resistant *An. gambiae* have been engineered in lab colonies, but techniques still need to be developed to release GM mosquitoes and drive anti-*P. falciparum* effector genes into wild *Anopheles gambiae* populations. *An. gambiae* has significant population structure, and reproductive barriers may hinder the spread of introduced genes, or require development of multiple strains for release into different populations. Within *An. gambiae*, the M and S molecular forms appear to be differentiated by three divergent speciation islands on the X, 2L, and 3L chromosomes. Although not physically linked, the speciation islands are nearly always found together, indicating that hybrids are selected against. Recent data shows that ecological conditions may lead to breakdown of reproductive barriers between the forms, resulting in brief periods of hybridization and introgression. Using a panel of SNPs located in each speciation island and the *kdr* insecticide-resistance allele, we genotyped ~700 *An. gambiae* samples from southern Mali, over a period spanning a hybridization and subsequent introgression event. We were able to track introgression of the *kdr-w* resistance allele from the S form into the M form. The *kdr-w* SNP is physically linked to the 2L island, but selection for the *kdr-w* allele was strong enough to overcome selection against hybrids, causing the S-form 2L island to introgress into the M form. These data indicate that despite strong reproductive barriers, advantageous *loci* are capable of introgressing between population groups during brief bursts of hybridization. Besides describing how alleles such as insecticide resistance can move between populations, this natural example of gene drive may help to develop effective methods to introduce engineered anti-malaria genes into field populations.

1118

BEHAVIORAL SWITCHES AND GENE REGULATION IN THE YELLOW FEVER MOSQUITO *AEDES AEGYPTI*

Luciano V. Cosme, Michel Slotman, Craig Coates

Texas A&M University, College Station, TX, United States

The yellow fever mosquito *Aedes aegypti* displays considerable behavioral and physiological changes after acquiring a blood meal. Unfed females actively seek humans, but suppress this behavior after blood feeding, and switch to looking for oviposition sites within 48 hours. These changes are expected to be correlated with the expression of the olfaction genes that play a crucial role in these behaviors. Over two hundred olfaction related genes have been identified in *Ae. aegypti*. To examine which of these may play a role in host seeking, we peformed RNA-seq to quantify changes in the expression of olfaction gene mRNA in the main olfaction organ of *A. aegypti*; the antennae. Four-day-old females were blood fed and antennae were collected 0, 3, 24, 48 and 72h after feeding. Four functional groups of genes were analyzed: Olfaction Receptors (Ors), Ionotropic Receptors (Irs), Odorant Binding Proteins (Obps), and Gustatory Receptors (Grs). Most of the Obps are highly expressed in the antenna of females, whereas most Grs are absent. The Irs are generally expressed at low levels. In contrast, many ORs are expressed at intermediate levels. The expression profile of Obps and Irs does not change remarkably post bloodmeal, compared to the Ors. Or46 and Or99 are only expressed in unfed females whereas Or49 is only expressed after feeding. Totally 14 Irs, 6 Ors and 6 Obps are upregulated after feeding, while 8 Irs, 4 Ors and 11 Obps are downregulated post feeding. To examine if miRNAs may play a role in the regulation of olfaction gene translation, we also examined the expression of miRNAs in female antennae 24h after feeding, revealing the presence of over 30 new miRNAs. We are conducting a bioinformatics and *in vitro* assay to determine the targets of these antennal miRNA genes. The discovery of miRNAs controlling key genes required for mosquitoes to complete their life cycle will not only help us to better understand the vectors' biology, but can also help the development of novel vector control tools.

1119

MINING *ANOPHELES GAMBIAE* MOSQUITO GENOME FOR RESISTANCE GENES AGAINST MALARIA

Xiaohong Wang[1], Genwei Zhang[1], Baolin Wu[2], John I. Githure[3], Guiyun Yan[4], **Jun Li**[1]

[1]University of Oklahoma, Norman, OK, United States, [2]University of Minnesota, Minneapolis, MN, United States, [3]International Centre for Insect Physiology and Ecology, Nairobi, Kenya, [4]University of California Irvine, Irvine, CA, United States

In order to identify genes related to *Plasmodium* parasite infection in *Anopheles gambiae* mosquitoes, we narrowed the *A. gambiae* parasite resistance island (PRI) to five small genomic blocks using co-expression patterns of genomic blocks, followed by direct association studies between non-synonymous single nucleotide polymorphisms (SNPs) and *Plasmodium falciparum* infection in wild *A. gambiae* populations from Kenya. The *A. gambiae* adenosine deaminase (AgADA), fibrinogen-related protein 30 (FBN30), and fibrinogen-related protein 1 (FREP1) genes were determined to be significantly associated with the *P. falciparum* parasite infection, and their functions in mosquitoes for the *Plasmodium* parasite infection were confirmed by RNA interference knockdown assays. When the FREP1 gene expression was knocked down, the *P. berghei* infection prevalence rate in *A. gambiae* mosquitoes were strikingly reduced from about 80% to 30%, indicating that the FREP1 gene is essential for *Plasmodium* invasion and may act as a receptor of *Plasmodium* parasites.

1120

SERUM MARKERS OF SEVERE CLINICAL COMPLICATIONS DURING *PLASMODIUM VIVAX* MALARIA MONOINFECTION IN THE PERUVIAN AMAZON BASIN

G. Christian Baldeviano[1], Karina P. Leiva[1], Antonio M. Quispe[1], Julio Ventocilla[1], L. Lorena Tapia[1], Salomon Durand[1], Meddly L. Santolalla[1], Leonila Ricopa[1], Karen Campos[1], Moises Sihuincha[2], Edward S. Smith-Nuñez[1], Kimberly A. Edgel[1], Andres G. Lescano[1]

[1]U.S. Naval Medical Research Unit - 6, Callao, Peru, [2]Hospital de Apoyo de Iquitos, Departamento de Medicina, Iquitos, Peru

Plasmodium vivax is the most widely distributed malaria species worldwide and responsible for ~75% of malaria cases in the Americas. There is growing evidence of severe complications caused by this malaria species. To identify biomarkers of severe malaria presentation caused by *P. vivax* mono-infection, we conducted a case-control study in two major reference hospitals in the city of Iquitos, Peru. Plasma samples were collected at time of malaria diagnosis from 46 subjects with *P. vivax* malaria mono-infection (severe malaria) who met at least one of a modified WHO criterion for severe malaria and 54 control subjects who presented with uncomplicated *vivax* malaria (non-severe malaria). Seventeen cytokines, chemokines and growth factors were assessed by Luminex xMAP technology and anti-$PvMSP1_{19}$-specific IgG antibodies were determined by ELISA. We found a significant elevation in the levels of interleukin (IL)-1β, IL-6, IL-10, IL-12, IL-13, IL-17, granulocyte macrophage colony-stimulating factor, gamma-interferon, monocyte chemotactic protein-1 and tumor necrosis factor alpha in the plasma of subjects with severe malaria ($p<0.05$). No significant difference was found in the levels of IL-2, IL-4, IL-5, IL-7, IL-8, granulocyte macrophage colony-stimulating factor and macrophage inflammatory protein 1 alpha. In addition, the titers of anti-$PvMSP1_{19}$-specific IgG antibodies were not significantly different between cases and controls, suggesting similar degree of prior malaria exposure in the two groups. Receiver operating characteristic (ROC) curve analysis revealed that IL-6 (AUC=0.76; 95%CI: 0.6-0.8) and IL-10 (AUC=0.73; 95%CI: 0.6-0.82) best discriminated severe from uncomplicated *vivax* malaria and, respectively) after adjusting for age, gender, *parasitemia* and levels of anti-PvMSP1 IgG antibodies. Further validation of these markers may provide accurate surrogate markers of severe complication during *vivax* malaria in endemic areas. We are currently investigating the predictive capacity of other inflammatory molecules including markers of endothelial cell activation and superoxide dismutase.

1121

DETAILED KINETICS OF B CELL SUBSETS FOLLOWING SYMPTOMATIC MALARIA IN CHILDREN IN TORORO, UGANDA

Richard T. Sullivan[1], Isaac Ssewanyana Ssewanyana[2], Sam Wamala Wamala[2], Charles Ebusu[2], Felistas Nankya[2], Eleanor Riley[3], Harriet Mayanja[4], Chris Drakeley[3], Mary Kakuru Muhindo[2], Emmanuel Arinaitwe[2], Jordan Tappero[5], Frank Kaharuza[6], Grant Dorsey[1], Bryan Greenhouse[1]

[1]University of California San Francisco, San Francisco, CA, United States, [2]Infectious Diseases Research Collaboration, Kampala, Uganda, [3]London School of Hygiene & Tropical Medicine, London, United Kingdom, [4]Department of Medicine, Makerere University College of Health Sciences, University of California San Francisco, Kampala, Uganda, [5]Global AIDS Program, Centers for Disease Control and Prevention, Atlanta, GA, United States, [6]Global AIDS Program, Centers for Disease Control and Prevention, Kampala, Uganda

Little is known about the kinetics of B cell subsets following naturally occurring human malaria infection. Recently, atypical memory B cells (MBC) have been hypothesized to have a role in protection. To evaluate these kinetics, we enrolled 40 children 4-5 years of age from an ongoing cohort study in Tororo, Uganda, where malaria transmission is intense (EIR >300). We collected blood via fingerprick at the time of symptomatic

malaria (D0), during which children were treated with an ACT, plus days 3, 7, 14, and 28 following treatment. Whole blood was stained, lysed, and frozen for subsequent analysis of B cell subsets via 10 color flow cytometry. Plasma cell frequencies were elevated on D0 (median=6.1% of B cells, IQR=3.5-8.8%), and decreased by D7 (median=2.5%, IQR=1.1-3.9%, p<0.005 vs. D0). Therefore, the day of presentation with symptomatic malaria provides a good time point to sample plasma cells in the periphery, many of which have likely differentiated due to the acute infection. Atypical MBC frequencies increased significantly from D0 (median=9.15%, IQR=5.8-12.5%) to D28 (median=15.8%, IQR=10.2-21.4%, p<0.001). Subjects with recently documented asymptomatic *parasitemia* had higher frequencies of atypical MBCs D0-D28 (p=0.049). Subjects with D0 parasite densities <25,000/ul also had higher frequencies of atypical MBCs D0-D28 (p=0.002). These data suggest a possible association between higher frequencies of atypical MBCs and that they may have a role in protection against malaria. Subjects with the highest incidence of malaria in the prior year (≥7 episodes/yr, n=20) exhibited a transient drop in frequencies of innate-like MBCs (CD27+IgD+IgM+) at the time of acute malaria not seen in subjects with a lower incidence (p<0.005 on D0 and D3, normalizing by D7). The reasons for this drop in innate-like MBCs, the primary contributor to serum IgM, in subjects with the highest incidence of malaria is unclear but warrants further investigation.

1122

A *FCGR2B* VARIANT THAT REDUCES HUMORAL IMMUNE SUPPRESSION IS ASSOCIATED WITH INCREASED SERUM MALARIA-SPECIFIC ANTIBODY LEVELS

Alexander Barron[1], Arlene Dent[1], Peter Siba[2], James W. Kazura[1], Christopher L. King[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]Papua New Guinea Institute of Medical Research, Goroka, Eastern Highlands Province 411, Papua New Guinea

Engagement of the FcγRIIb receptor by antigen-containing immune complexes is a critical negative regulator of host immune responses on monocytes, macrophages and B cells. Recent observations show that homozygosity for a *FCGR2B* mutation that dampens inhibitory signaling (I→T at amino acid residue 232) is associated with increased risk for autoimmune diseases, but is also associated with protection against severe malaria in Kenyan children. Protection against severe malaria in 232T/T Kenyan children may result from a more robust humoral immune response against blood stage malaria and/or enhanced phagocytosis, which may select for this mutation. This hypothesis is supported by observations that the 232T/T variant is highest in malaria endemic areas (7-11%) and low elsewhere (~1-2%). We found 232T/T frequencies of 9 and 18% in Hardy-Weinberg equilibrium in two malaria endemic Papua New Guinea (PNG) populations whereas 232T/T was 6% in a PNG highland population not known to be associated with malaria endemicity. All Papuan populations have significantly higher 232T/T and 232I/T frequencies than Caucasians from North America and Northern Europe (p<0.0001 to 0.006) indicating a potential founder effect. However, the 232T/T and 232I/T genotypes are more frequently found in one malaria endemic PNG population than in the non-malaria endemic highlands population (p=0.027) suggesting a role for malaria in maintenance of the 232T allele in this malaria-endemic Papuan group. Surprisingly, 232T/T adults (>=18yo) had lower antibody (Ab) levels to a panel of most *Plasmodium vivax* (Pv) *P. falciparum* (Pf) Ags compared to individuals with 232I/I and 232I/T adjusted for age (p=0.0019 to 0.0488). Regression models of the age-related acquisition of anti-Pv and -Pf Ab responses indicate that 232T/T diverge from 232I/I and 232I/T between 4-10yo (Pv) or 6-20yo (Pf). Enhanced phagocytosis of opsonized malaria-infected erythrocytes in subjects with 232T/T may sustain this mutation in populations with heavy malaria and other infection burdens.

1123

IS SEROLOGICAL CROSS-REACTIVITY PREDICTED BY GENE ORTHOLOGY? A PROTEIN MICROARRAY STUDY OF SEROLOGICAL CROSS-REACTIVITY BETWEEN *APICOMPLEXA*N PARASITES *PLASMODIUM FALCIPARUM, P. VIVAX* AND *TOXOPLASMA GONDII*

Sultan Gulce Iz[1], Aarti Jain[1], Douglas M. Molina[2], Christopher Hung[1], Li Liang[1], Christian Baldeviano[3], Andres G. Lescano[3], Kimberly A. Edgel[3], Peter Crompton[4], Mert Döşkaya[5], Joseph Vinetz[6], Philip Felgner[1], Yüksel Gürüz[5], **D. Huw Davies**[1]

[1]University of California Irvine, Irvine, CA, United States, [2]Antigen Discovery Inc., Irvine, CA, United States, [3]U.S. Naval Medical Research Unit - 6, Lima, Peru, [4]National Institutes of Health/National Institute of Allergy and Infectious Diseases, Rockville, MD, United States, [5]Ege University, Bornova/İzmir, Turkey, [6]University of California San Diego, San Diego, CA, United States

Antibodies are known to be exquisitely specific recognition molecules, and dysfunction of this property may have serious consequences for immune function and autoimmunity. Nevertheless, cross-reactivity of a given antibody with two or more related antigens does occur. The rules that define cross-reactivity are not well understood but are assumed to relate to amino-acid sequence homology between two or more antigens recognized by a given antibody. To examine cross-reactivity experimentally we constructed protein microarrays displaying proteins from three apicomplexan parasites: *Plasmodium falciparum*, *P. vivax*, and *Toxoplasma gondii.* Orthologous genes were identified using the *'Transform by Orthology'* tool implemented in EuPathDB (eupathdb.org). This uses the OrthoMCL database in which orthologs are defined by a combination of phylogeny-based, evolutionary distance-based and BLAST-based algorithms. Thus, *P. falciparum* and *P. vivax* share 5,259 orthologous genes, while *T. gondii* shares 3,114 and 2,980 orthologs with *P. falciparum* and *P. vivax*, respectively. Protein microarrays were probed with sera from human malaria cases from Mali (exclusively *P. falciparum*) and Peru (predominantly *P. vivax)* and from toxoplasmosis cases and controls from western Turkey (the only *P. vivax* cases in Turkey are in the east). In addition to detecting specific antibodies as expected, *P. falciparum* arrays detected antibodies in *P. vivax* infections from Peru, and *P. vivax* arrays detected antibodies in *P. falciparum* infections from Mali. These results show the arrays are able to detect cross-reactivity. Interestingly, antibody cross-reactivity between *P. falciparum* and *P. vivax* was observed for both orthologous and non-orthologous proteins. In contrast, toxoplasmosis sera showed only weak reactivity to *P. falciparum* or *P. vivax* antigens and were distributed equally among the cases and controls. These data show that orthology alone is not a good predictor of serological cross-reactivity.

1124

DETERMINATION OF THE IDEAL TIME TO MEASURE CYTOKINE EXPRESSION IN CULTURED PBMCS FROM *PLASMODIUM FALCIPARUM* INFECTED PATIENTS FROM IQUITOS, PERU

Katherin Peñaranda[1], Pamela Rodriguez[1], Katherine Torres[1], Joseph Vinetz[2], Dionicia Gamboa[1]

[1]Universidad Cayetano Heredia, Lima, Peru, [2]University of California San Diego, San Diego, CA, United States

Peripheral blood mononuclear cells (PBMCs) are used as an *in vitro* model for immunogenetic research with the stimulation of antigens. Different protocols use different incubation periods assuming that the dynamic in time of cytokines expression is similar. Theoretically, cytokine expression begins 6 hours after stimulation, but each cytokine expression vary. This study aimed to know the incubation period needed to obtain the highest cytokine expression in PBMCs non-stimulated and stimulated with MSP1 from malaria patients and endemic controls. Three cryopreserved PBMCs samples obtained by leukapheresis were used: one control (C), one asymptomatic (AS) and one symptomatic (S) patient. Each sample was

incubated at 37°C for 6, 10, 14, 18, 22 and 26 hours (h) after stimulation with MSP1 and non-stimulated. The cells then were collected for RNA extraction and cDNA synthesis. The mRNA from 4 cytokines was tested: TNF-α, IFN-γ, IL-10 and IL-12 by qPCR. The highest expression of cytokines was found between 6 and 14h in C and AS patients and between 14 and 26h in S patients. For Th1 cytokines (IFN-γ and TNF-α) the expression at 6 and 10h was similar between the S and the AS samples, being lower than the control and less expressed than the housekeeping gene. However, we found a high expression of IFN-γ and TNF-α at 14h in S sample with a relative expression of 10 and 4 folds higher than the control respectively. Compared to AS sample the relative expression of IFN-γ and TNF-α was 32 and 2.25 folds higher respectively. For IL-12, the relative expression at 6 to 18h is higher in the C than in patients, but at 26h the S is 2.25 folds higher than the others. For IL-10, the expression between 6 and 10h is higher in the C than in patients and between 14 and 26h S and AS expression is higher than the C, being more expressed at 18h in the AS. With these preliminary results we could infer that the ideal time to measure Th1 and Th2 cytokine expression in cultured PBMCs is within the first 14h or after 18h respectively.

1125

HOST AND PARASITE FACTORS UNDERLYING ASYMPTOMATIC *PLASMODIUM FALCIPARUM* INFECTION IN MALIAN CHILDREN

Silvia Portugal[1], Aboudramane Bathily[2], Jeff Skinner[1], Shanping Li[1], Tuan M. Tran[1], Safiatou Doumbo[2], DIdier Doumtabe[2], Younoussou Kone[2], Seydou Dia[2], Moussa Niangaly[2], Charles Darra[2], Jules Sangala[2], Ogobara K. Dombo[2], Kassoum Kayentao[2], Aarti Jain[3], D. H. Davies[3], Christopher Hung[3], Li Liang[3], Philip L. Felgner[3], Zbynek Bozdech[4], Manuel Llinas[5], Aissata Ongoiba[2], Boubacar Traore[2], Peter D. Crompton[1]

[1]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [2]Malaria Research and Training Center, Bamako, Mali, [3]University of California Irvine, Irvine, CA, United States, [4]Nanyang Technological University, Nanyang, Singapore, [5]Princeton University, Princeton, NJ, United States

In Mali, *Plasmodium falciparum* transmission is seasonal during the 6-month rainy season, when almost all children below 8 years of age experience one or more febrile malaria episodes. On the other hand, during dry season virtually no children experience symptomatic malaria, even though ~50% carry blood-stage parasites. To determine the host and parasite factors that underlie asymptomatic infection, we performed a one-year longitudinal study of 580 children between 1 and 11 years of age. Subjects infected with *P. falciparum* by rapid diagnostic testing (RDT) at the end of the dry season were treated with standard anti-malarial drugs while RDT negative but PCR positive individuals for *P. falciparum* were left untreated; the third group included those who were uninfected at the end of the dry season. The risk of *P. falciparum* infection and clinical malaria was assessed prospectively during the subsequent malaria season and immune parameters were compared over time in the three groups. Individuals infected with *P. falciparum* at the end of the dry season who were left untreated showed a decreased risk of clinical malaria during the ensuing malaria season compared with uninfected individuals, and interestingly, treatment of asymptomatic infection at the end of the dry increased the subsequent risk of febrile malaria compared to the untreated group, indicating that chronic asymptomatic infection protects from febrile malaria. Analyzing polymorphic regions of *P. falciparum* genes we also compared the risk of super-infection in children whose asymptomatic infection at the end of the dry season was treated or not. In addition we are examining parasite factors that underlie chronic asymptomatic infection by comparing the metabolome and transcriptome of parasites collected from asymptomatic children *versus* parasites collected from children with acute febrile malaria. These data provide valuable insights into the host and parasite factors that underlie asymptomatic *P. falciparum* infection, as well as the risks associated with treating chronic asymptomatic *P. falciparum* infection.

1126

STUDIES OF *PLASMODIUM FALCIPARUM*-ASSOCIATED MODULATION OF THE HOST IMMUNE RESPONSE

Jacqueline Moebius[1], Silvia Portugal[1], Aissata Ongoiba[2], Kassoum Kayentao[2], Safiatou Doumbo[2], Didier Doumtabe[2], Younoussou Kone[2], Ogobara K. Doumbo[2], Boubacar Traore[2], Peter D. Crompton[1]

[1]National Institutes of Health, Rockville, MD, United States, [2]Malaria Research and Training Center, Department of Epidemiology of Parasitic Diseases, Faculty of Medicine, Pharmacy and Odonto-Stomatology, University of Bamako, Mali, Mali

T helper cells play a critical role in orchestrating effective antibody responses, yet little is known about the CD4 T cell response to *Plasmodium falciparum* infection in humans. Recent data from animal models indicate that *Plasmodium* infection induces functional $CD4^+$ T cell exhaustion, and that reversal of $CD4^+$ T cell exhaustion leads to enhanced B cell and antibody responses and accelerates clearance of blood stage parasites, thus offering a potential explanation for the inefficient acquisition of protective antibodies in response to *P. falciparum* infection in endemic areas. In longitudinal studies in Mali in which PBMCs are collected before, during and after symptomatic and asymptomatic *P. falciparum* infections, we are testing hypotheses related to *P. falciparum*-associated modulation of the host immune response such as T cell exhaustion. In preliminary studies we observed that *P. falciparum* infection results in a higher frequency of CD4 T cells expressing programmed death-1 (PD-1), an inhibitory receptor associated with functional exhaustion of T cells. In ongoing work we are extending these analyses to understand the impact of acute and chronic *P. falciparum* infection on the phenotype and function of other components of the adaptive and innate immune system.

1127

MALARIA DURING PREGNANCY

Maria F. Yasnot[1], Douglas J. Perkins[2], Jaime Carmona-Fonseca[3], Prakasha Kemprai[2], Mauricio Corredor[4], Amanda Maestre[3]

[1]Grupo de Investigaciones Microbiológicas y Biomédicas de Córdoba, Universidad de Cordoba, Monteria, Colombia, [2]University of New Mexico, Albuquerque, NM, United States, [3]Grupo Salud y Comunidad, Universidad de Antioquia, Medellín, Colombia, [4]Grupo GEBIOMIC, Universidad de Antioquia, Medellin, Colombia

Malaria during pregnancy is a leading cause of maternal and infant morbidity, due to mother´s anemia, early birth and low birth weight. In Latin America, few studies address the subject of the immune response during gestational malaria, this is more notorious in *Plasmodium vivax* infection. The aim of the current study was to establish the effect of *P. vivax* infection in the balance of pro- versus anti- inflammatory cytokines and chemokines, and their relationship with some clinical and epidemiology outcomes. To this end, 43 pregnant women inhabitants in Uraba-South Cordoba. Out of these, 15 subjects were included at delivery (MGP+), 8 had history of gestational malaria (MGP-) and 20 had no exposition to infection throughout the pregnancy. Epidemiology and clinical data (age, gestational age, number of previous pregnancies, newborn´s weight, mother´s hemoglobin and parasitaemia) were recorded after reviewing the clinical records. At delivery, whole blood and plasma from the mother, placenta and cord, as well as placental tissue were collected. Diagnosis of infection was performed by thick smear and real time PCR. Pro-inflammatory ((TNFα, IFNγ, IL1β, IL17)/anti-inflammatory (IL4, IL6, IL10, TGF-β) cytokines and chemokines were measured by real time PCR and plasma level of them were assessed by multiplex ELISA. In order to characterize the changes on placental tissue (cell infiltrates, parasites, hemozoin, among other), histopathology analysis was performed and identification of the monocytes and NK cell infiltrates was carried out by immunohistochemistry. The clinical and epidemiology variables explored were similar in the three groups with exception of the gestational age. Placentas from the infected groups evidenced histopathology changes

including chronic villitis, fibrin deposition, intervillous inflammation, and hemozoin deposition. Monocyte and NK cells infiltrates were observed within the intervillous space and in villi itself. Cytokine expression showed a bias towards a pro-inflammatory status in both groups. Anti-inflammation cytokines remained unchanged. MCP1 was high in placentas of the MGP+ group and IL8 was high in the MGP- group. In conclusion, *P. vivax* induced modulation towards pro-inflammatory cytokines in placentas and produced histopathology changes that might affect the mother and fetus.

1128

ANTIBODY PROFILING IN INDIVIDUALS WITH *VIVAX* MALARIA LIVING IN TWO AREAS WITH DIFFERENT MALARIA ENDEMICITY AND DISEASE SEVERITY IN THE PERUVIAN AMAZON

G. Christian Baldeviano[1], Huw Davies[2], Aarti Jain[2], Li Liang[2], Chris Hung[2], Juan F. Sanchez[1], Salomon Durand[1], Edward S. Smith-Nuñez[1], Philip Felgner[2], Joseph Vinetz[2], Kimberly Edgel[1], Andres Lescano[1]

[1]U.S. Naval Medical Research Unit - 6, Lima, Peru, [2]Division of Infectious Diseases, Department of Medicine, University of California, Irvine, CA, United States

The development of naturally acquired immunity to *Plasmodium falciparum* malaria in endemic populations is believed to be affected by transmission intensity, age and parasite diversity based on studies conducted in high-transmission settings in Africa. However, very little is known regarding the acquisition of antibodies against *P. vivax*, particularly in areas of moderate to low malaria transmission. In addition, it is unclear whether individuals with different malaria clinical presentation differ in their anti-parasite antibody profile. To examine these issues, we measured the levels of IgG antibodies against ~500 *P. falciparum* and ~500 *P. vivax* seroreactive proteins (approximately ~10% of the malaria proteome) using protein microarray. We included individuals with PCR-confirmed *P. vivax* monoinfection who were enrolled in two areas with distinct malaria epidemiology and clinical presentation: Iquitos (IQT, n=20) and Madre de Dios (MDD, n=20), which are located in the Peruvian Amazon Basin. Among subjects from IQT, we included 9 cases who were hospitalized with *vivax* malaria and 11 non-hospitalized *vivax* malaria patients. Our preliminary results showed that the overall antibody reactivity to *P. vivax* proteins tended to be higher in individuals from IQT compared to those in MDD; about 3% of these differences reached statistical significance ($p<0.05$). Antibody responses against *P. falciparum* proteins were higher in Loreto where 10% the cases are due to *P. falciparum* malaria compared to Madre de Dios where no cases of *P. falciparum* have been reported in the last 10 years. Furthermore, uncomplicated malaria cases consistently presented higher levels of antibody against both *P. vivax* and *P. falciparum* compared to hospitalized malaria cases. Our results suggest that the degree of malaria endemicity, previous exposure to *P. falciparum* and potentially disease severity may influence the profile of anti-*P. vivax* antibodies generated during infection in low-to-moderate transmission settings.

1129

COMPARISON OF TRANSFORMATION, NORMALIZATION AND TESTING CHOICES IN A PROTEIN MICROARRAY ANALYSIS PIPELINE

Jeff Skinner, Peter D. Crompton

National Institutes of Health, Rockville, MD, United States

Protein microarrays have established an important role in the study of antibody responses to many tropical infectious diseases including brucellosis, malaria, melioidosis and salmonellosis. Papers have been published on appropriate methods for processing and analyzing protein microarray data, but few cover the entire procedure from start to finish or make fair comparisons among the various processing and analytical alternatives. We have categorized the processing of protein microarray data into three distinct steps: transformation, normalization and background subtraction. We also present several statistical testing options to compare the breadth and magnitude of antibody profiles. We then compare several transformation, normalization and statistical testing methods using previously published protein microarray data in which *Plasmodium falciparum*-specific antibody profiles were examined in a longitudinal cohort study in Kambila, Mali. We find robust linear model (RLM) normalization with generalized linear model tests of the antibody profile breadth to reveal the most powerful insights into the immune response against malaria. Going forward, establishing standard approaches to processing and analyzing protein microarray data will improve the rigor of antibody profiling studies and will facilitate cross-study comparisons.

1130

DIFFERENTIAL RESPONSE OF IFN-Γ USING DIFFERENT ANTIGENS IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF *PLASMODIUM FALCIPARUM* ACUTE MALARIA PATIENTS BY ELISPOT ASSAY: PRELIMINARY RESULTS

Claudia Carrera[1], Elizabeth Villasis[1], Katherine Torres[1], Joseph Vinetz[2], Dionicia Gamboa[1]

[1]Cayetano Heredia Peruvian University, Lima, Peru, [2]University of California San Diego, San Diego, CA, United States

This project has the aim to determine the production of IFN-γ by peripheral blood mononuclear cells (PBMCs) associated to clinical data in patients with *Plasmodium falciparum* acute malaria of different communities from Maynas, Peru. A total of 10 patients from rural (communities near to Nanay River) and peri-urban areas (Punchana and San Juan), and 3 endemic controls from an urban area (Iquitos) were tested in an IFN-γ ELISpot assay expressed by Spot-forming cells (SFC) per 2x105 cells. PBMCs were stimulated with 3 antigens: hemozoin (Hz), AMA-1 protein and merozoites (Mz). Hz and Mz were obtained from two different strains, ITG (referential strain) and F06 (wild isolate). We also used Hz DNase-treated as a control. We observed a difference between IFN-γ response to Hz ITG (23 SFC/2x105 PBMCs) and Hz F06 (26 SFC/2x105 PBMCs) versus Hz ITG DNase-treated (18 SFC/2x105 PBMCs) and Hz F06 DNase-treated (12 SFC/2x105 PBMCs) however there was no statistical difference. Mz ITG and Mz F06 were 68 SFC/2x105 PBMCs and 56 SFC/2x105 PBMCs, respectively. There was no statistical significance. The mean spots for AMA-1 protein was 3 SFC/2x105 PBMCs, very similar to the negative control. There was no significant difference between ITG and F06 strains in the IFN-γ response for Hz and Mz. We also reported a difference between genders in the IFN-γ production, men presented high levels of IFN-γ in Hz ITG, Hz F06, Mz F06 and AMA-1. Although Mz ITG presented a greater response for women, the difference is not significant comparing with men response. Furthermore, the *parasitemia*s (parasites/uL) were higher in men (12183p/uL) than women (3483p/uL). We only find a weak correlation ($R^2 = 0.4034$) between levels of IFN-γ and *parasitemia* in men, in which high concentrations of IFN-γ tend to have low levels of *parasitemia*. The 3 endemic controls did not present a significant IFN-γ response to the antigens. In conclusion, Mz presented the highest IFN-γ response in all studied patients followed by Hz. Unfortunately; AMA-1 recombinant protein presented the lower response of IFN-γ. Moreover, this study suggests that gender is a possible associated factor to the development of immune response and that IFN-γ production is related to *parasitemia* levels indicating that the immune system is acting against the parasite.

1131

PRELIMINARY EVALUATION OF CYTOKINES GENE EXPRESSION IN SYMPTOMATIC VERSUS ASYMPTOMATIC PATIENTS INFECTED WITH *PLASMODIUM FALCIPARUM* IN THE PERUVIAN AMAZON

Pamela Rodriguez[1], Katherine Torres[1], Dionicia Gamboa[1], Joseph Vinetz[2]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]University of California San Diego, San Diego, CA, United States

The balance between Th1 and Th2 cytokines seem to be crucial to survive an infection which requires the generation of a controlled immune response in the host, which recognizes and eliminates the invading pathogen limiting collateral damage to tissues, which may be an exacerbated immune response. The aim of this study was to evaluate the gene expression profile of pro-inflammatory Th1, regulatory cytokines and a chemokine in symptomatic versus asymptomatic *Plasmodium falciparum* infected patients from the Peruvian Amazon, in order to identify the cytokine pattern displayed by them during the acute disease. mRNA levels of Peripheral blood mononuclear cells (PBMCs) was analyzed in *P. falciparum* patients with different clinical outcome (Symptomatic, asymptomatic and control) which were cultured with different stimuli (Hemozoin (Hz), Hemozoin treated with DNase (HzTx), CpG and the MSP-1 recombinant protein). Within symptomatic individuals we have observed high levels of expression of TNF-α, IFN-γ and IL-12 (Th1 Cytokines) being predominant in PBMC's that were stimulated with Hz and moderate with HzTx. Likewise, TLR9 presented high levels of gene expression, the same pattern was observed when were stimulated with Hz and CpG. These levels of cytokine correlated with specific symptoms shown in each individual and *parasitemia* levels. These results were entirely different in the asymptomatic individual, finding very low levels of Th1 cytokines but presented moderate levels of RANTES gene expression when was stimulated with Hz but lower levels with HzTx. In this patient, we also observed high levels of IL-10 gene expression when was stimulated with MSP-1. In addition, other immunosuppressive activity related molecules were evaluated (CTLA4, FoxP3) and we found that the levels of expression of both molecules were lower in symptomatic compared to asymptomatic. In conclusion, we have observed that there is a characteristic cytokine expression profile that is involved in specific clinical status: Symptomatic samples presented high Th1 cytokines gene expression and asymptomatic presented moderate to high regulatory cytokines gene expression.

1132

OWNERSHIP AND USE OF INSECTICIDE-TREATED BEDNETS IN MACHINGA DISTRICT, MALAWI, SIX MONTHS AFTER A MASS-DISTRIBUTION CAMPAIGN

Don P. Mathanga[1], Monica Shah[2], Dyson Mwandama[1], Laura Steinhardt[2], Doreen Ali[3], John Zoya[3], Exton Mtande[1], Andy Bauleni[1], Kim Lindblade[1]

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

Insecticide-treated bednet (ITN) mass distribution campaigns are being used to rapidly achieve ITN universal coverage and increase utilization..As part of the national mass distribution campaign in 2012, every household in six villages in Machinga district, Malawi, received one long-lasting polyester ITN for every two people in the household plus one extra for odd-numbered households. Existing bed nets were not taken into account in determining the number of ITNs to be given in the campaign. To evaluate the impact of the campaign on ITNownership and usage, two household censuses were conducted in February and November 2012, four months before and six months after the campaign. During each census, we collected data on bednet ownership and usage, sleeping spaces, demographics of household members and household characteristics. During the February and November census, 2,200and 2,657 households were censused and interviewed, respectively. Household ownership of at least one ITN increased from 70% (95% confidence interval [CI] = 68%-72%) before the campaign to 84% (95% CI = 83%-86%) afterwards. The averagenumber of ITNs per household increased from 1.1 to 1.8. Households with at least one ITN for every two residents increased from 23%(95% CI = 22%-25%) to 56% (95% CI = 54%-58%). ITN usage in all residents rose from 58% (95% CI = 57 - 59) to 70% (95% CI = 69 - 71). The percentage-point increase in ITN use was highest amongst school-aged children (between 5 and 15 years old),from 49% to 66%,whereas children aged <5 years increased from 71% to77% and adults >15 years increased from 59% to 70%. The mass campaign greatly improved ITN ownership and use although the goal of one ITN per two residents was not achieved. This could be because of the time lag between censuses and the campaign. Use of ITNs among school-age children continues to lag behind other age groups.

1133

TAKING LOCAL OWNERSHIP: GOVERNMENT AND HOUSEHOLD CONTRIBUTIONS TO INDOOR RESIDUAL SPRAYING FOR MALARIA CONTROL IN ZANZIBAR AND MAINLAND TANZANIA

Zainab Alidina[1], Shabbir Lalji[1], Joshua Mutagahywa[1], Mahdi Ramsan[1], Jessica M. Kafuko[2], Uche Ekenna[3], Stephen Magesa[4], Jeremiah Ngondi[1], Rajeev Colaco[3]

[1]Research Triangle Institute, Dar es Salaam, United Republic of Tanzania, [2]President's Malaria Initiative, Dar es Salaam, United Republic of Tanzania, [3]Research Triangle Institute, Durham, NC, United States, [4]Research Triangle Institute, Nairobi, Kenya

While foreign assistance has been instrumental in the initiation and implementation of large-scale malaria control efforts in Tanzania and other African countries, national government contributions are key to local ownership and sustainability. Indoor Residual Spraying (IRS) interventions were started in Zanzibar and Mainland Tanzania in 2006 and 2007, respectively, primarily through funding from the United States President's Malaria Initiative. This study aimed to explore in-kind contributions of the local government and households towards the total costs of IRS interventions. Data were collected through detailed interviews with local government officials involved in IRS and the technical team for the IRS project. Household contribution was estimated based on provision of water for IRS by households. Government contributions included government-provided warehouse and office space, fuel, vehicles and staff labor. In-kind cost contributions were analyzed and aggregated at the district, regional and national level. Calculations were based on proportion of total costs of IRS for the years 2008, 2009 and 2010. The cost per structure sprayed in Mainland Tanzania was $14.54 in 2009 and reduced to $14.23 in 2010. Zanzibar had a lower cost per structure sprayed; $9.78 and $6.92 in 2008 and 2010, respectively. On average, total in-kind contribution was 4.5% in Zanzibar and 3.0% in Mainland Tanzania. The proportion of government in-kind contribution was higher in Zanzibar versus the Mainland (3.4% vs. 1.3%) while household contribution was higher in Mainland Tanzania compared to Zanzibar (1.7% vs. 1.1%). As a proportion of total IRS expenditure, there was no increase in in-kind contribution in Mainland Tanzania or Zanzibar between 2008 and 2010. Government involvement, particularly through monetary transactions and increased in-kind contribution, needs to be encouraged for malaria control efforts to be locally owned, managed and sustained. To increase government and household contribution towards IRS, the government needs to become more centrally involved in project support, financing and capacity building.

1134

A COST-EFFECTIVE, PRACTICAL AND SCALABLE COMMUNITY-BASED MOSQUITO TRAPPING SCHEME THAT CAPTURES SPATIAL AND TEMPORAL HETEROGENEITIES OF MALARIA TRANSMISSION IN RURAL ZAMBIA

Chadwick H. Sikaala[1], Aklilu Seyoum[2], Gerry F. Killeen[2]

[1]Ministry of Health, Lusaka, Zambia, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Understanding factors contributing to spatial and temporal heterogeneity of malaria transmission at community level is important in determining control measures to apply. However, to promote sustainability and ownership of malaria control at community level, there is need for developing practically affordable sampling schemes. Community-based Centres for Disease Control and Prevention miniature light trap (CDC-LT) and the Ifakara Tent Trap model C (ITT-C) were compared with controlled quality assured (QA) human landing catch (HLC), CDC-LT and ITT in 14 clusters randomly selected for long lasting insecticidal nets alone or supplemented with organophosphate or pyrethroid -based indoor residual spraying (IRS). Cost and practical implication of the sampling methods were taken into account. Parasite and sporozoite rates were measured using trained local community health workers (CHW). There was no significant difference (p>0.05) in the mean catches of *Anopheles funestus* Giles by the community -based scheme (CBS) and the QA one. The crude relative sensitivity of ITT to the CDC light trap was 0.397. None of the methods captured more mosquitoes than the quality assured HLC indoor. Both HLC indoor and outdoor were equally efficient (Relative rate [95% Confidence Interval] = 1.104 [1.091, 1.210]).Parasite rates were variable over all the clusters but consistently high during the wet season with the organophosphate-based IRS clusters being lower than the rest. Similarly, *An.funestus* sporozoite infectivity rates followed a similar pattern. The CBS was relatively cheaper than the central level driven one. CBS systems could be affordable and reliable alternatives for surveillance of malaria in resource constrained malaria control programs. Even though HLC was more efficient than any other sampling tool, its ethical issues and laborious nature remain stumbling blocks for consideration at community. While both the parasite positivity rates and the sporozoite infectivity are consistently high in the wet season and in clusters which were not supplemented with organophosphate-based IRS, results in one cluster suggest this intervention requires periodic application to lower the saturated entomologic inoculation rate to levels were impact will be appreciated.

1135

ESTABLISHMENT OF AN *ANOPHELES DARLINGI* COLONY UNDER INSECTARY CONDITIONS THROUGH NATURAL COPULATION IN IQUITOS, PERU

Cuauhtemoc Villarreal[1], Gissella M. Vasquez[2], Karin Escobedo-Vargas[3], Anibal Huayanay-Repetto[3], Victor Lopez-Sifuentes[3], Carmen Flores-Mendoza[2], Andres G. Lescano[2], **Frederick M. Stell**[2]

[1]Centro Regional de Investigación en Salud Pública/Instituto Nacional de Salud, Tapachula, Chiapas, Mexico, [2]U.S. Naval Medical Research Unit - 6, Lima, Peru, [3]U.S. Naval Medical Research Unit - 6, Iquitos, Peru

Anopheles darlingi is the main malaria vector in the Americas and among the most important in the world. Yet, very few studies describe its bionomics, parasite-vector relationships and vector competence mainly due to the lack of a laboratory colony. Important constraints limiting *An. darlingi* rearing have been the inability to stimulate copulation and oviposition in artificial environments, and the limited knowledge on optimal larvae rearing. We describe the establishment of an *An. darlingi* colony through manipulation of environmental conditions that promote natural copulation and the implementation of methods that effectively induce oviposition and reduce mortality. Adult females were collected in the village of Zungarococha, Department of Loreto, Peru, and taken to the NAMRU-6 Insectary in Iquitos where F0 offspring was reared. F0 adults (4,230) were placed in cages (60x60 cm) and stimulated to copulate naturally under a thermoperiod of 30°C during the day and 25°C at night and with a 30 min LED light stimulation period at dusk. Different oviposition containers were evaluated to optimize egg-laying. Larvae feeding regime and rearing conditions were standardized. We have used these conditions to successfully rear *An. darlingi* up to F3. An average of 36 sexual encounters/day/cage was observed in the first two generations ranging from 4-100 throughout a 5-day stimulation period. Spermatheca dissections (N=760 females) indicated an average insemination rate of 49% with the highest rate (95%) observed 8 days after stimulation. A total of 7,993 F1 eggs and 10,590 F2 eggs were obtained. F3 egg (11,000) and larvae (5,000) production is ongoing successfully. The average egg to adult mortality was 5% in the F1 (7,593 adults) and 63% in the F2 (3,873 adults). Pupae to adult mortality was low (0-4%) in both generations. Our novel, effective *An. darlingi* rearing methodology may allow critical studies of this important vector and could be a generalizable, cost-effective alternative to forced mating, a labor-intensive method generally used in anopheline laboratory rearing.

1136

HOUSEHOLD COSTS OF INPATIENT MANAGEMENT OF MALARIA IN MALAWI

Joseph D. Njau[1], Melissa Briggs[1], Jobiba Chinkhumba[2], Andy Bauleni[2], Monica Shah[1], A. Chalira[3], D. Moyo[3], W. Dodoli[4], Meshack Luhanga[3], John Sande[3], Doreen Ali[3], Don Mathanga[2], Kim Lindblade[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Malawi College of Medicine, Blantyre, Malawi, [3]Ministry of Health, Blantyre, Malawi, [4]World Health Organization, Blantyre, Malawi

Little is known of the economic burden on households caused by costs of treatment for severe malaria. We present household costs resulting from inpatient treatment of severe malaria in Malawi, where 65% of the population live on less than $1 a day. We implemented a nationally representative cross-sectional survey of inpatient malaria management and costs. Thirty-six health facilities admitting malaria patients were randomly selected from a national list of hospitals stratified by managing authority and location. Researchers spent two days at each facility and interviewed all patients admitted for malaria within six hours prior to arrival. Patients being discharged on the day of interview were asked questions on hospital (consultation, laboratory tests, drugs and admission fees) and non-hospital costs (transport, accommodation and meals for patients and caregivers) incurred, before and during their admission. Costs incurred during previous health care visits for the same illness were included in the totals presented. A total of 82 patients were enrolled, of whom 46 (56%) were female and 42 (51%) were children under five years of age. The mean length of admission was 2.3 nights (range 0.5 to 12 nights). Nine (11%) patients reported seeking care at another facility prior to admission. Only 10 (12%) patients reported no expense during treatment of their illness. Of the remaining 72 patients, the overall mean expenditure was US$ 9.00 with a median of US$ 3.40 and a range of US$ 0.11 to US$ 144.81. Average hospital costs were higher (US$ 13.36) than non-hospital costs (US$4.57, p<0.011). On average, admissions at hospitals managed by the Christian Health Association of Malawi (CHAM) were more expensive (US$ 14.23) relative to those at hospitals managed by the government (US$5.23, p<0.010). Given the poverty level in Malawi, the inpatient treatment costs for severe malaria cases were high and potentially catastrophic to the majority of households. This study presents additional evidence and dimensions of the economic burden of malaria placed on poor households.

1137

FEASIBILITY, SAFETY AND EFFECTIVENESS OF COMBINING HOME BASED MALARIA MANAGEMENT (HMM) AND SEASONAL MALARIA CHEMOPREVENTION (SMC) IN CHILDREN LESS THAN TEN YEARS IN SENEGAL: A CLUSTER-RANDOMIZED TRIAL

Roger C. Tine[1], Cheikh T. Ndour[2], Babacar Faye[1], Magatte Ndiaye[1], Khadime Sylla[1], Doudou Sow[1], Jean L. Ndiaye[1], Oumar Gaye[1]

[1]Service de Parasitologie, Faculté de Médecine de Dakar, Dakar, Senegal, [2]Clinique des Maladies Infectieuses, Centre Hospitalier Universitaire de Fann, Sénégal, Dakar, Senegal

Current antimalarial strategies recommend (i) prompt access to diagnostic testing and treatment with effective antimalarial drugs such as Artemisinin Combination Therapy (ACT), (ii) intermittent preventive treatment for pregnant woman and infant (iii) Seasonal Malaria Chemoprophylaxis (SMC) and (iv)impregnated bed nets. Combination of antimalarial interventions can significantly reduce malaria burden. However, limited information is available on mechanism of combining antimalarial interventions. This operational research was conducted to assess the feasibility and effectiveness of introducing an integrated malaria control strategy, including HMM (using RDT, ACTs, Rectal artesunate) and SMC delivered by community health workers (CHWs). A cluster-randomised trial was carried out during 2 transmission seasons (2010-2011) in 8 villages located in the Southeastern part of Senegal. The intervention arm was represented by the combination of HMM+SMC while HMM represented the control arm. Children <10years of age were weekly followed by CHWs. At each visit, axillary temperature was measured and children with malaria suspected fever were RDT tested. Primary end point was the incidence of malaria attack over the follow up period. Secondary end points included: (i)malaria diagnostic accuracy (ii)access to ACT treatment (iii)SMC coverage (iv)safety and drug tolerability. The adjusted rate ratio comparing incidence of malaria attacks in intervention communities with control communities was 0.15, indicating a protective effect of HMM+SMC at 85%, (95%CI[21.2%-97.1%], p=0.005). RDT sensitivity and specificity respectively represented 89.2%(95%CI:70-100) and 86.2% (95%CI:70.2-100). A proportion of 96.4% of RDT confirmed malaria attack received AL. SMC coverage was evaluated at 97.3% (95%CI:91.3-100) in 2010, and 94.1% (95%CI [89.3-99.2] in 2011. No serious adverse event was noted. This study provided evidence that it is feasible to deliver SMC alongside an effective HMM intervention, while achieving high coverage and effectiveness of both SMC and HMM.

1138

MYANMAR ARTEMISININ RESISTANCE CONTAINMENT (MARC) SURVEY: MALARIA DIAGNOSIS AND TREATMENT

Celine Zegers de Beyl[1], Myat Phone Kyaw[2], **Ohnmar Hlabaw**[2], Thae Maung Maung[2], Sylvia Meek[1], Krongthong Thimasarn[3], Aye Yu Soe[4], David Sintasath[5], Thar Tun Kyaw[6]

[1]Malaria Consortium, London, United Kingdom, [2]Department of Medical Research (Lower Myanmar), Yangon, Myanmar, [3]World Health Organization, Yangon, Myanmar, [4]Three Diseases Fund, Yangon, Myanmar, [5]Malaria Consortium, Bangkok, Thailand, [6]Vector Borne Disease Control, Ministry of Health, Nay Pyi Taw, Myanmar

The emergence and potential spread of artemisinin resistance in Cambodia, Thailand, Vietnam and Myanmar calls for immediate action from the global public health community to eliminate artemisinin resistant parasites from the region. One of the pillars of the Myanmar Artemisinin Resistance Containment (MARC) strategy is to ensure timely and effective case management of all malaria cases. However, limited epidemiological data available in Myanmar pose a challenge to monitor progress in stemming this global public health threat. In 2012, a malaria household survey was conducted in the areas of known and suspected artemisinin resistance (Tier 1 and Tier 2) to serve as a baseline for the MARC. The study domains included representative populations living in high to moderate malaria risk areas and utilized a multi-stage sampling approach stratified by Tier. In total, 1992 household respondents were interviewed using standardized and pre-tested questionnaires in line with similar malaria surveys previously conducted in Cambodia and Thailand. Overall, only 2.6% (95%CI 1.7 to 3.9) respondents would seek confirmation of malaria with microscopy or rapid test and 28.9% (95%CI 25.0 to 33.2) were able to name any specific antimalarial drug. According to the survey, the public sector was cited as the most popular source for test and treatment for malaria in both Tiers (66%); however, more in-depth analysis of this and a separate health facility survey are needed to better delineate public-private sources. Awareness of appropriate diagnostic services and treatment for malaria was insufficient in the MARC areas, and it is concerning that only a few respondents knew that some antimalarials were not recommended. Better targeted and innovative behavior change communications are needed to improve malaria knowledge and treatment-seeking behaviors amongst community members living in containment areas while also working with public and private (regulated and nregulated) providers to ensure provision of quality care for the diagnosis and treatment of malaria.

1139

A COMPARATIVE COST-EFFECTIVE ANALYSIS STUDY FOR ADOPTING UNIVERSAL MALARIA RAPID DIAGNOSTIC TESTS (MRDT) IN PEDIATRIC FEVERS ACROSS THREE SUB-SAHARAN AFRICA COUNTRIES

Joseph D. Njau[1], Stephen P. Kachur[1], Deborah McFarland[2]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Rollins School of Public Health, Emory University, Atlanta, GA, United States

In 2010, the World Health Organization issued a new guideline for malaria treatment. The guideline called for universal testing of malaria parasites prior to treatment in patients of all ages. Debates on whether malaria testing should be adopted for children under5 have divided malaria researchers. Economic evidence on the effectiveness of adopting such a strategy especially in settings with variable malaria transmission intensities remains sparse. This study explores whether adoption of Rapid Diagnostic Tests (mRDT) for malaria treatment in children under-five would be cost effective relative to presumptive treatment strategy in areas with variable malaria epidemiology across sub-Saharan Africa. We used Malaria indicator survey (MIS) data from three countries with different malaria transmissions to determine the effectiveness of the strategy. Additionally, country specific cost data on mRDT toolkits, drugs as well as health seeking behavior for children under5 and clinical practices were gathered from government agencies and also from published and unpublished studies. In cases where data were missing, experts' opinions were used. Data were uploaded in TreeAge decision tree software to determine the costs and cost-effectiveness of using mRDT relative to presumptive malaria treatments. Adoption of mRDT strategy for malaria treatment in children under5 was found to be cost effective across the three study countries relative to presumptive treatment strategy. However, the threshold for mRDT effectiveness was variable across the three countries. For instance, adoption of mRDT strategy in Angola was found to be highly cost effective compared to Uganda or Tanzania which had lowest prevalence cut-off points for the mRDT strategy being cost-effective. Local factors such as malaria prevalence, cost of testing supplies, the volumes of patients seeking care at primary health facilities and clinician's compliance were critical in determining the level of mRDT cost-effectiveness relative to presumptive treatment strategy. Universal formulation of malaria policies may not necessarily be cost effective. Policies should be tailored to reflect country specific malaria transmission status, clinical practices and local economic conditions.

1140

EXPLORING PATHWAYS THROUGH WHICH MATERNAL EDUCATION RELATES TO CHILDHOOD MALARIA INFECTIONS

Joseph D. Njau[1], Stephen Kachur[1], Rob Stephenson[2], Deborah McFarland[2]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Rollins School of Public Health, Atlanta, GA, United States

A number of studies have shown some strong relationships between maternal education child health and survivorship. There are very few studies that have systematically investigated the pathways and the interactions between maternal education and childhood malaria infections. We present evidence on the relationship between maternal education and childhood malaria infections based on cross-sectional malaria indicator survey data from three sub-Saharan Africa countries. We used pooled malaria indicator survey data from three sub-Saharan Africa countries and estimated multivariate logistic regression models to determine the relationship between maternal education and childhood malaria infections. We report the marginal effects for the probabilities of maternal education relating to childhood malaria infections. Additionally, we performed the Oaxaca decomposition analysis to quantify the contribution of maternal education on childhood malaria infections. The full adjusted model showed significant statistical association between maternal education and childhood malaria infection. Children under the age of five years within households where their mothers reported having some primary education level were 3.2 percentage points ($p<0.01$) less likely to have malaria parasites. Meanwhile, children of mothers with education level beyond primary school were 4.7 percentage points less likely to be malaria positive ($p<0.001$). The Oaxaca decomposition analysis of the full adjusted model exhibited a 7.8% gap in childhood malaria infection rates between educated and uneducated mothers. 60.8% of exhibited gap was largely explained by differences in household wealth, household place of domicile and differences in regional malaria transmission intensities. There was a significant statistical relationship between maternal education and childhood malaria infections across the three study countries. These findings provide additional evidence for support of malaria control policies focusing on integrated sector-wide approach for sustainable malaria control programs. Maternal education has huge potentials for sustainable long term reductions of childhood malaria infections reductions.

1141

TESTING MARKET-BASED AND REGULATORY STRATEGIES TO MINIMIZE OVERTREATMENT WITH ANTI-MALARIAL DRUGS IN ZANZIBAR

Alexandra Morris[1], Abdullah Ali[2], Bruno Moonen[1], Mwinyi Msellem[2], Abdul-wahiyd Al-mafazy[2], Abdunoor Mulokozi[3], Abigail Ward[1], Laura Kelley[1], Anne Wilson[1], Justin Cohen[1]

[1]Clinton Health Access Initiative, Boston, MA, United States, [2]Zanzibar Malaria Control Programme, Zanzibar, United Republic of Tanzania, [3]Ifakara Health Institute, Dar es Salaam, United Republic of Tanzania

Malaria burden in Zanzibar is at an all-time low, but the 2011 malaria indicator survey suggests only about one quarter of children receive blood tests for febrile illness, meaning that many who receive anti-malarials may not truly need them. Newly low-endemic regions like Zanzibar require strategies for ensuring that anti-malarial drugs go to those who truly need them. This study evaluated an intervention to improve rational use of anti-malarials in Zanzibar in which regulatory authorities implemented a policy banning sales of anti-malarials from over-the-counter (OTC) shops while subsidised malaria rapid diagnostic tests (RDTs) were introduced in private health facilities. Testing was already widely available in public health facilities. A controlled pre-post evaluation was used to assess whether the intervention changed the proportion of those with suspected malaria who sought care in OTC shops and of those that received parasitological confirmation. It also evaluated the impact of the regulations on the proportion of OTC shops selling anti-malarials. A midline evaluation was conducted six months after the baseline to determine the short-term effects of the intervention, and an endline evaluation was conducted a full year following implementation. Each evaluation consisted of 1,000 household surveys, 119 mystery client surveys, and 66 provider surveys. After six months, the intervention had a significant effect on the removal of antimalarials from OTC shops: the proportion of OTCs stocking antimalarials fell from 85% at baseline to 22% at midline (Chi2 Test $p<0.001$). However, the fraction of suspected malaria cases seeking treatment in OTC shops and receiving diagnosis remained unchanged at six months. The final evaluation conducted in April 2013 was analyzed to assess the impact of the intervention one year after regulations began, including qualitative investigation of patient perceptions of the new regulations. The results of this project suggest that government regulation of the private anti-malarial market is a viable path to encourage proper case management in an elimination setting.

1142

LOW-COST EDUCATIONAL TRAINING CAUSES SUSTAINED IMPROVEMENT IN PRESCRIBING AND STOCKING OF ARTEMISININ-BASED DRUGS IN MADAGASCAR

Abigail Ward, Alexandra Morris, Pierre-Loup Lesage, Aimée Miller, Felix Lam, Justin Cohen

Clinton Health Access Initiative, Boston, MA, United States

Although artemisinin-based combination therapies (ACTs) are the recommended treatment for malaria in Madagascar, the 2011 malaria indicator survey indicated that fewer than 40% of anti-malarial drugs received by children under five for fever were ACTs. Approximately one third of suspected malaria is treated in the private sector where ACTs are often unaffordable or inaccessible. To encourage prescribing, stocking, and purchasing of ACTs in the private sector of Madagascar, educators were deployed to share scientifically accurate knowledge about ACT effectiveness with doctors and shopkeepers. Baseline cross-sectional surveys on factors related to prescription practices, anti-malarial stocking, and consumer drug preferences were conducted in 234 outlets and 163 health facilities in five regions of Madagascar in July 2011. Doctors and outlets in intervention regions were then visited by trained educators with ACT outreach materials between October 2011 and March 2012. Follow-up surveys in all regions were conducted in April 2012 immediately after the intervention concluded and again eight months later to evaluate longer-term effects. Midline results indicated that shops not stocking ACTs at baseline were 113% ($chi2=17.6$, $p<0.01$) more likely to do so if visited by educators, while doctors who had never prescribed them were 107% ($chi2=5.9$, $p<0.02$) more likely after receiving the educational visits. At endline, eight months after the visits ended, 93.8% of shops that began to stock ACTs and 92.3% of doctors who began prescribing them continued to do so. A 122% increase in anti-malarial market share of ACTs was observed in exit interviews with 406 customers at midline in both groups (27.7-60.4% intervention; 37-69.8% control), likely due to AMFm implementation in October 2010. Market share was similar at midline and endline (58.2% intervention; 69.7% control, n=391). These results suggest the potential for a short-term, low-cost intervention to cause sustained improvements in the availability of recommended drugs.

1143

DOCUMENTING MALARIA CASE MANAGEMENT COVERAGE IN ZAMBIA WITH A SYSTEMS EFFECTIVENESS APPROACH

Megan Littrell[1], John M. Miller[2], Micky Ndhlovu[3], Busiku Hamainza[4], Chibesa Wamulume[4], Davidson H. Hamer[5], Richard W. Steketee[1]

[1]Malaria Control and Evaluation Partnership in Africa (MACEPA), PATH, Seattle, WA, United States, [2]Malaria Control and Evaluation Partnership in Africa (MACEPA), PATH, Lusaka, Zambia, [3]Chainama College of Health Sciences, Lusaka, Zambia, [4]National Malaria Control Centre, Ministry of Health, Lusaka, Zambia, [5]Center for Global Health and Development, Boston University; Zambia Center for Applied Health Research and Development, Lusaka, Zambia

National malaria control programs and their partners must document progress associated with investments in malaria control. While this has been achieved through population-based surveys for most interventions, measuring changes in malaria case management practices has been challenging because the increasing use of diagnostic tests often reduce the denominator of febrile children who should be treated with an ACT - thus the indicator "proportion of fever cases in young children treated with the recommended antimalarial drug within 24 hours of onset" is less relevant. We examined an alternative sequence of indicators using a systems effectiveness approach and data from nationally representative surveys in Zambia: the 2012 population-based malaria indictor survey (MIS) and the 2011 health facility survey (HFS). The MIS measured treatment-seeking behavior among 972 children under five (CU5) and 1848 people age 5+ with recent fever. The HFS assessed case management of 435 CU5 and 429 people age 5+ with fever or history of fever seeking care at 149 health facilities. Consultation observation and exit interviews measured use of malaria diagnostic tests, ACT prescription, and patient comprehension of prescribed regimens. Systems effectiveness, using MIS and HFS data, was estimated as follows: 100% ACT efficacy x 47% fever treatment seeking from an appropriate provider x 68% blood testing x 88% ACT prescription for positive cases x 67% full patient comprehension of the drug regimen for patients prescribed ACT (proxy for adherence) = 19%. The largest gap in systems effectiveness in this context is low levels of treatment seeking behavior for fever. However, MIS results show that about half of febrile children who did not seek care from an appropriate provider had no evidence of infection (blood slide and RDT negative). Assembling MIS and HFS data to benchmark progress that can be attributed to investments in scale up of malaria diagnostics and treatment is feasible and can guide decision making to further improve malaria case management.

1144

MALARIA IN MPUMALANGA, SOUTH AFRICA: A MATHEMATICAL MODELING APPROACH TO UNDERSTANDING TRANSMISSION

Sheetal P. Silal[1], Francesca Little[1], Karen I. Barnes[1], Lisa Jane White[2]

[1]University of Cape Town, Rondebosch, Cape Town, South Africa, [2]Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand

Malaria has been and remains a significant threat in Mpumalanga, a province on the Mozambican border of South Africa. As Mpumalanga is considered to be in the malaria elimination phase and the South African government begins to intensify efforts and commit scarce resources to decrease malaria incidence, there is a need to understand patterns in malaria transmission so that efforts may be targeted appropriately. Mathematical models have in the past provided a valuable framework for analyzing the dynamics of malaria transmission and are increasingly being used to test policy interventions so as to determine their impact on simulated transmission before implementing interventions in the field. Malaria transmission in Mpumalanga is seasonal from September to April, but transmission is still unstable and prone to sporadic outbreaks. The source distribution has changed in the last decade with 60% of reported cases being locally sourced in 2002, while at the end of 2012, 87% of reported cases had a foreign source; the vast majority originating from Mozambique. The season is characterized by three peaks where locally sourced infections and the northern most portion of the province contribute to the first peak (Spring) and foreign-sourced infections dominate the second two peaks (Christmas and Easter). While time series techniques showed rain lagged at 5 weeks to be significantly correlated with reported cases, multiple regression did not find rain as a significant covariate; a result that makes geographical sense given the trend of more foreign-sourced cases. An ordinary differential equation model of transmission is also used to validate these findings. Being able to understand the nature and source of malaria transmission will enable policy-makers to develop appropriate antimalarial strategies and this may lead to a better allocation of scarce resources and ultimately a greater impact on malaria.

1145

MODELING DEMAND FOR ARTEMISININ COMBINATION THERAPIES BASED ON EMPIRICAL RETAIL PRICES AND TREATMENT-SEEKING DATA FROM HOUSEHOLD AND OUTLET SURVEYS

Aaron M. Woolsey[1], Justin M. Cohen[1], Stephen Poyer[2], Bruno Moonen[1]

[1]Clinton Health Access Initiative, Boston, MA, United States, [2]Population Services International, Nairobi, Kenya

Global efforts to increase uptake of artemisinin combination therapies (ACTs) have focused on improving access and reducing the cost of ACTs across all distribution channels, most recently through the efforts of the Affordable Medicines Facility for malaria. However, reductions in the retail price of ACTs have had unclear effects on their uptake, and while broad distribution of ACTs has improved their accessibility, cheaper (though less effective) alternative antimalarials remain widely available. Since 2009, ACTwatch has conducted surveys at treatment access points in 8 sub-Saharan African countries: Benin, Democratic Republic of the Congo, Kenya, Madagascar, Nigeria, Uganda, Zambia, and Zanzibar. These biannual surveys collect volume and price data for all antimalarial medicines over the course of a week. For each treatment access point in the surveys, we calculated the fraction of antimalarial sales that were ACTs (mean <7% across all countries) and modeled this outcome as a function of mean ACT sales prices relative to mean sales prices of other available antimalarial drugs. By controlling for potentially confounding covariates (e.g., outlet type, access point accessibility, population density, and malaria prevalence), we modeled an empirical relationship describing how ACT market share changes with retail price. Results confirm that lower prices were associated with greater uptake. For example, ACT prices were 5.5x more expensive than non-ACTs in Nigerian shops that did not sell any ACTs during the 2011 survey period, but only 3.5x more expensive in shops that did sell ACTs. Applying the derived relationship to survey-derived estimates of demand for antimalarial drugs across sub-Saharan Africa yields estimates of ACT demand at different price points, providing information that drug manufacturers can use for production planning and giving policy makers a critical tool to shape the market dynamics of current and future malaria treatment interventions.

1146

INCREMENTAL COST-EFFECTIVENESS ANALYSIS OF INTRODUCING RAPID DIAGNOSTIC TESTING FOR MALARIA INTO REGISTERED DRUG SHOPS IN UGANDA

Kristian S. Hansen[1], Anthony K. Mbonye[2], Sham Lal[1], Pascal Magnussen[3], Sian E. Clarke[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Ministry of Health, Kampala, Uganda, [3]University of Copenhagen, Copenhagen, Denmark

Universal access to diagnostic testing for malaria followed by artemisinin-based combination therapy (ACT) for positive cases is now recommended by WHO. It is common in Uganda for people to seek treatment for malaria outside the formal health care sector often with drug shops as their first choice. Parasitological diagnosis to guide malaria treatment is not usually offered in drug shops. A recently finalised cluster-randomised intervention trial in Mukono District, Uganda, demonstrated that testing with malaria rapid diagnosis tests (mRDTs) in drug shops is feasible, can be associated with high provider compliance and resulted in significant increase in appropriate treatment compared to presumptive treatment. The incremental cost-effectiveness analysis of this intervention was evaluated using a decision analytical approach. Societal costs were collected for the two study arms. Provider costs incorporated the cost of community sensitisation, training of drug shop vendors, development of training material and the commodity costs such as the mRDTs used and the ACTs dispensed. Household costs of health care seeking were captured in a sample of drug shop customers who were interviewed in their homes after their initial visit to a drug shop. The purpose of these home interviews was to obtain all household cost incurred during a two-week period after the drug shop visit. These costs included out-of-pocket expenditure for travelling, fees, diagnosis and drugs for the first and any subsequent treatment visits as well as the opportunity cost of lost time. The effectiveness measure was 'correctly treated patient' defined as a research blood slide positive patient receiving an ACT or a blood slide negative patient not receiving an ACT. Effects were also translated into Disability-Adjusted Life Years lost. The incremental cost and effects of introducing mRDTs to increase appropriate ACT treatment in private, registered drug shops will be presented followed by sensitivity analyses incorporating factors like adherence, provider compliance and accuracy of the test.

1147

NOVEL LIVER STAGE ANTIGENS ASSESSED AS POTENTIAL VACCINE CANDIDATES AGAINST MALARIA

Alexander Pichugin[1], Lindsey Ehrler[1], Zachary MacMillen[2], Theresa Funk[1], Cate Speake[2], Bob Morrison[2], Patrick Duffy[3], Urszula Krzych[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Seattle BioMed, Seattle, WA, United States, [3]National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States

The most attractive target for vaccine development against malaria is pre-erythrocytic liver stage (LS) of infection. The major barrier in developing vaccines is identification of antigens that will stimulate the most effective immune responses - a key for long-lasting protection. In this study we have screened and selected novel effective pre-erythrocytic vaccine candidates. On the basis of previously identified by microarray and RNA sequencing analyses *P. falciparum* (Pf) genes expressed exclusively during LS of infection, we selected *P. berghei* (Pb) orthologues of Pf genes and validated their expression by qRT-PCR in Pb infected C57Bl/6 (B6) mice. DNA constructs for 23 selected genes were cloned and tested for protective efficacy in B6 mice by Gene Gun, Electroporation and IM delivery. The efficacy of each DNA vaccine candidate was tested in a challenge model with CSP-DNA as positive control. Protective efficacy was measured by the reduction of Pb18S rRNA at 40h after challenge. 11 Pb LS antigens, administered as DNA vaccines, significantly reduced LS parasite burden in mice infected with Pb sporozoites and the level of reduction varied from 30% to 80% relative to vaccination with an empty vector. Two antigens sustained protection during 6 months after the last immunization. Moreover, three novel Pb LS antigens improved protective effect of CSP-DNA in our experimental model. Although we demonstrated that antibody responses induced by novel liver stage DNA antigens well correlated with the reduction of liver-stage parasite burden, depletion of CD8+ T cells resulted in loss of protective effects of DNA vaccines. Hence, our preliminary data suggest that protection induced by our novel pre-erythrocytic antigens was mediated by CD8+ T cells. This strategy allowed us to compare and prioritize new antigens according to their ability to induce partial protective immunity against malaria in mice. The protective antigens are being delivered by more robust vector platforms to achieve sterile protection and the results from these studies will be discussed.

1148

IDENTIFICATION OF NEW AND HIGHLY PROTECTIVE PRE-ERYTHROCYTIC ANTIGENS FOR MALARIA VACCINE DEVELOPMENT

Joseph T. Bruder[1], Ping Chen[1], Greg Ekberg[1], Bennett Myers[1], Christopher Lazarski[1], Emily Smith[2], Joao Aguiar[2], Keith Limbach[2], Noelle B. Patterson[2], Martha Sedegah[2], Thomas Richie[2], Eileen Villasante[2], Denise L. Doolan[3], Doug L. Brough[1]

[1]GenVec, Gaithersburg, MD, United States, [2]Naval Medical Research Center, Silver Spring, MD, United States, [3]The Queensland Institute of Medical Research, Brisbane, Australia

Malaria is the most devastating parasitic disease affecting humans. There is a great need for an effective malaria vaccine that could provide robust protection and contribute to the control and eventually eradication of malaria. Pre-erythrocytic antigens are particularly promising targets for malaria vaccine development with great potential to prevent infection and transmission, however, very few of these antigens have previously been identified and these are not sufficient to confer high levels of protection. Our vaccine rationale is based on the fact that immunization with radiation-attenuated sporozoites (RAS) or live sporozoites with chloroquine prophylaxis (spz + CQ) provide high level (~100%) protection against sporozoite challenge in both mice and humans, and this protection is dependent on the induction of CD8+ T cells targeting multiple antigens expressed in the pre-erythrocytic stages of the parasite life cycle. Our goal was to identify these novel antigens. Using a high-throughput genomics screening approach, we have identified some of the antigens that are targets of protective T cell responses in mice immunized with RAS and spz + CQ. Several of these antigens induced high levels of protection in outbred mice challenged with *Plsmodium yoelii* sporozoites. Our antigen discovery system utilizes an array of adenovirus vectors expressing 300 highly expressed *P. yoelii* pre-erythrocytic genes with identifiable *P. falciparum* orthologues. In the antigen discovery screen, antigen presenting cells were infected with individual adenovectors from the array and then mixed with splenocytes from mice immunized with protective regimens of RAS. We prioritized antigens based on the frequency of CD8+ T cell recall responses to each of the 300 antigens. We selected 50 antigens that recalled the most robust T cell responses and tested their capacity to protect mice from a *P. yoelii* sporozoite challenge. Outbred CD1 mice were immunized with a DNA prime - Ad boost regimen and sterile protection was measured following sporozoite challenge. Several of the prioritized antigens induced high levels of sterile protection. The *P. falciparum* orthologues of these antigens are being considered for advancement to clinical development.

1149

VACCINATION WITH SCHIZONT EGRESS ANTIGEN-1 PROTECTS MICE FROM *PLASMODIUM BERGHEI* ANKA CHALLENGE

Dipak K. Raj[1], Christian P. Nixon[1], Hai-Wei Wu[2], Grant Jolly[3], Lauren Pischel[1], Ailin Lu[1], Christina Nixon[1], Ian Michelow[2], Ling Cheng[1], Jennifer F. Friedman[2], Michel Fried[4], Patrick E. Duffy[4], **Jonathan D. Kurtis**[5]

[1]Center for International Health Research, Rhode Island Hospital, Brown University Medical School, Providence, RI, United States, [2]Center for International Health Research and Department of Pediatrics, Rhode Island Hospital, Brown University Medical School, Providence, RI, United States, [3]Department of Pathology and Laboratory Medicine, Rhode Island Hospital, Brown University Medical School, Providence, RI, United States, [4]Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [5]Center for International Health Research and Department of Pathology and Laboratory Medicine, Rhode Island Hospital, Brown University Medical School, Providence, RI, United States

We discovered PfSEA-1 using a differential screening approach contrasting plasma from children who were resistant or susceptible to *falciparum* malaria. Antibodies to the immunorelevant region of PfSEA-1 (rPfSEA-1A, aa 810-1083) predict resistance to severe disease in two yr old children and block schizont egress from iRBCs. To evaluate the protective efficacy of vaccination with SEA-1 *in vivo*, we selected the *Plasmodium berghei* ANKA model based on its aggressive parasite growth rate, extreme lethality and the failure of known vaccine candidates (i.e. AMA-1 and MSP-1) to afford protection. We expressed and purified the *P. berghei* ANKA strain ortholog of rPfSEA-1A in *E. coli*, and conducted three vaccine trials in mice. Following vaccination, mice generated robust anti-rPbSEA-1A IgG responses in each trial. In Trial 1, BALB/c mice were vaccinated IP with rPbSEA-1A in TiterMax and challenged IP with 106 *P. berghei* ANKA iRBC. In Trial 2, C57BL/6 mice were vaccinated SC with rPbSEA-1A in TiterMax and challenged with 200 *P. berghei* ANKA sporozoites IV and in Trial 3, BALB/c mice were vaccinated IP with rPbSEA-1A in TiterMax and challenged IP with 104 *P. berghei* ANKA iRBC. In all three experiments, rPbSEA-1A conferred marked protection against a typically lethal *P. berghei* ANKA challenge as evidenced by up to 3.05 fold reduction in *parasitemia* seven days post-challenge ($P < 0.001$). Control mice required euthanasia by day 7-8 post challenge due to high *parasitemia* with associated morbidity, while none of the vaccinated mice had high *parasitemia* or overt morbidity. In addition, vaccination with rPbSEA-1A resulted in self-cure in 1/11 vaccinated mice in the first trial. These data constitute the first report of protection in *P. berghei* ANKA by vaccination with blood stage antigens and support our ongoing efforts to evaluate PfSEA-1 in the Aotus model of *falciparum* infection using both active vaccination with rPfSEA-1 as well as passive transfer of anti-rPfSEA-1A monoclonal antibodies.

1150

ANTIBODIES TO *PLASMODIUM FALCIPARUM* GLUTAMIC ACID RICH PROTEIN (PFGARP) INHIBIT PARASITE GROWTH BY ARRESTING TROPHOZOITE DEVELOPMENT

Dipak K. Raj[1], Christina Nixon[1], Sunthorn Pond-Tor[1], Hai-Wei Wu[2], Jennifer F. Friedman[2], Michel Fried[3], Patrick E. Duffy[3]

[1]Center for International Health Research, Rhode Island Hospital, Brown University Medical School, Providence, RI, United States, [2]Center for International Health Research and Department of Pediatrics, Rhode Island Hospital, Brown University Medical School, Providence, RI, United States, [3]Laboratory of Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

In previous vaccine discovery efforts, we developed a differential screening method using plasma from children who were resistant or susceptible to *falciparum* malaria. Using this approach, we discovered PfSEA-1. Antibodies to PfSEA-1 predict resistance to severe disease in two yr old children, block schizont egress from infected RBC *in vitro*, and vaccination with rPbSEA-1A protects mice from *Plasmodium berghei* ANKA challenge. We have now adapted our differential screening method to field parasite-derived phage display libraries. In differential bio-panning assays, PfGARP (aa 411-673) was recognized by plasma pooled from resistant (n=11) but not susceptible (n=14) children participating in our birth cohort in Muheza, Tanzania. To further characterize PfGARP, we generated mouse antibodies against this immuno-relevant, highly invariant region (PfGARP-A) and performed growth inhibition (GIA) and immunolocalization studies. For GIA, 3D7 parasites were synchronized to the ring stage and plated at 0.3-0.4% *parasitemia* in the presence of anti-PfGARP-A or pre-immune sera (1:10 dilution). Parasites were cultured for 48 hrs and ring and early trohozoite stage parasites were enumerated. Anti-PfGARP-A inhibited parasite growth by 99% compared to controls ($P < 0.001$). In confocal studies, PfGARP localized to the RBC membrane in trophozoite and early schizont infected RBCs, but not to other parasite stages or uninfected RBC. To determine the mechanism of growth inhibition we performed trophozoite arrest assays (TAA) using anti-PfGARP-A. For TAA, 3D7 parasites were synchronized to the ring stage and plated at 5% *parasitemia* in the presence of anti-PfGARP-A or pre-immune mouse sera (1:10 dilution). Parasites were cultured for 36 hrs and trophozoite stage parasites were enumerated. Anti-PfGARP-A arrested trophozoite progression by 99% compared to controls ($P < 0.001$). These data support Pf-GARP as a novel vaccine candidate for pediatric *falciparum* malaria. By blocking trophozoite development, PfGARP may synergize with vaccines targeting hepatocyte and red cell invasion and schizont egress.

1151

DISCOVERY OF CONSERVED *PLASMODIUM* ANTIGENS ON THE SURFACE OF MALARIA-INFECTED RED BLOOD CELLS USING DNA APTAMERS

Eugene K. Oteng

University of Oxford, Oxford, United Kingdom

Malaria continues to present a major human and economic challenge causing over 1.2 million deaths in 2010 and billions in lost economic potential. A vaccine targeting the leading causative agents, *Plasmodium falciparum* and *P. vivax*, would reduce much of the poverty associated with malaria and greatly assist in eradication efforts. Yet despite decades of work, no licensed malaria vaccine exists and some of the few candidates that have been evaluated elicit strain specific responses due to extensive polymorphism. During its intraerythrocytic stages (IE), *P. falciparum* remodels the host red cell membrane with a complex and poorly defined assortment of parasite-encoded proteins that undergo antigenic variation. Despite the requirement for immunologic stealth, exported parasite proteins also mediate strain-independent functions such as endothelial sequestration that are critical for pathogenesis. Based on this observation, we hypothesized that *P. falciparum* displays novel structurally conserved proteins on the IE surface and these proteins may serve as antigens for a broadly effective anti-malarial vaccine. In order to test this hypothesis, we developed an *in vitro* evolution technique that sequentially incorporates unique *P. falciparum* isolates as the target for Systematic Evolution of Ligands by EXponential enrichment (Serial-SELEX) to generate nucleic acid molecular probes, aptamers, capable of recognizing conserved surface determinants. Of the 11 aptamers identified, 10 demonstrated parasite-specific binding with nanomolar dissociation constants. Examination of the binding specificity of radiolabeled aptamers revealed a subset of aptamers that recognized all laboratory-adapted clones and clinical isolates of *P. falciparum* tested. Remarkably, these aptamers also recognized all tested laboratory and clinical isolates of *P. vivax* and *P. knowlesi* but not the murine malaria parasites, *P. chabaudi* and *P. berghei*. Competition studies showed that the aptamers bound a single target which was confirmed as an IE membrane protein using biochemical techniques and confocal microscopy. Aptamer-mediated affinity purification and tandem mass spectrometry enabled the identification of the likely aptamer target.

Discovery of a protein conserved between the major human malarias may have implications for vaccine development and validates the Serial-SELEX technique as a powerful tool for antigen discovery.

1152

ENHANCING ANTIBODY IMMUNOGENICITY OF TRANSMISSION-BLOCKING MALARIA VACCINES

Sumi Biswas[1], Yuanyuan Li[1], Kazutoyo Miura[2], Sara E. Zakutansky[1], Carole A. Long[2], Robert E. Sinden[1], Simon J. Draper[1], Fergal Hill[3], Adrian V. Hill[1]

[1]The Jenner Institute, Oxford, United Kingdom, [2]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases/ National Institutes of Health, Rockville, MD, United States, [3]Imaxio, Lyon, France

Transmission blocking malaria vaccines (TBVs) target *Plasmodium falciparum* sexual stages, aiming to block their further development within the mosquito. Different delivery systems, targeting Pfs25, a leading TBV candidate antigen have previously shown good transmission-blocking efficacy in pre-clinical models but these results have not translated in humans. One of the major challenges in translating pre-clinical efficacy of TBVs to humans has been the apparent need for exceptionally high antibody titres against known targets to achieve good transmission blocking activity. It has been shown that the IC50 (antibody concentration required to inhibit 50% of parasites) of anti-Pfs25 antibodies are 15.9, 4.2, 41.2, and 85.6 µg/mL for mouse, rabbit, monkey and humans respectively, and the differences among species are significant. To improve the Pfs25 antibody response we have fused Pfs25 to IMX313, a short DNA sequence which spontaneously forms a heptamer (PMID: 18474650). This increases the antibody levels by a log compared to Pfs25 alone after immunisation of mice with viral-vectors and improves transmission-blocking activity. We have set up the Pichia pastoris protein expression system as a viable GMP-compatible platform for production of monomeric Pfs25 and Pfs25-IMX313 fusion. Our results indicate that the heptamerisation improves the immunogenicity after protein-in-adjuvant immunisation as well. Immunogencity and transmission-blocking efficacy results will be presented.

1153

EVALUATION OF CANDIDATE *PLASMODIUM FALCIPARUM* VACCINE ANTIGENS USING THE RADIATION ATTENUATED SPOROZOITE VACCINE MODEL

Jessica S. Bolton[1], Joyce A. Wanga[1], Emily C. Smith[1], Eileen F. Villasante[2], Thomas L. Richie[2], Joao C. Aguiar[3]

[1]Henry M. Jackson Foundation/Naval Medical Research Center, Silver Spring, MD, United States, [2]Naval Medical Research Center, Silver Spring, MD, United States, [3]CAMRIS International/Naval Medical Research Center, Silver Spring, MD, United States

To aid development of a pre-erythrocytic subunit vaccine protecting against *Plasmodium falciparum* malaria, we sought to evaluate antigen-specific immune responses of human volunteers immunized with radiation attenuated *P. falciparum* sporozoites (RAS). Volunteers were exposed to RAS by the bites of irradiated, *P. falciparum* infected mosquitoes every four weeks for a total of seven immunization sessions. PBMCs and sera were acquired by leukapheresis following the final immunization. Subsequently, volunteers were challenged by mosquito-bites of *P. falciparum*-infected mosquitoes. A panel of 21 candidate pre-erythrocytic antigens was selected for evaluation. To first evaluate the development of antibodies targeting each candidate antigen, protein was produced using the wheat germ cell-free expression system (Cell Free Sciences) and serum reactivity was evaluated by Western blot. Of 21 candidate antigens evaluated, 20 antigens were recognized by the sera of one or more volunteers. To evaluate memory T cell reactivity to these candidate antigens, cultured ELISpot was performed. Briefly, PBMCs were stimulated with HLA-predicted peptides and/or pools of overlapping 15mer peptides for 12 days, washed, and restimulated for 18 hours. The number of interferon-gamma producing T cells was evaluated by ELISpot. Of the 21 candidate antigens screened, 15 were recognized by PBMCs of at least one volunteer and 6 were negative for all volunteers. We observed a trend toward increased breadth of antigens recognized by individuals who were protected from *P. falciparum* challenge compared to unprotected individuals, although this did not reach statistical significance. Antigens demonstrating reactivity among protected individuals have been prioritized for further evaluation and development as candidate vaccine antigens.

1154

GENERATION OF "FULLY HUMAN" MONOCLONAL ANTIBODIES TO *PLASMODIUM FALCIPARUM* FROM HUMAN-IMMUNE-SYSTEM HUMANIZED (DRAG) MICE

Yuliya Kleschenko[1], Andrea Akyeampong[1], Sai Majji[1], Wathsala Wijayalath[1], Kathy Moch[1], Jason Regules[1], Megan Dowler[1], Robert Gerbasi[1], Eileen Villasante[1], Thomas L. Richie[1], Teodor-Doru Brumeanu[2], Sofia Casares[1]

[1]Naval Medical Research Center/Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Uniformed Services University of the Health Sciences, Bethesda, MD, United States

Fully human monoclonal antibodies (mAb) are envisioned as a new therapeutic approach for neutralization of infectious agents/toxins, while devoid of side effects associated to the use of mouse, chimeric, or humanized antibodies. The current challenge for generation of fully human mAbs is the paucity of specific B cells in human blood, since antibody-secreting plasma cells reside in lymphoid organs and bone marrow. Approaches that use EBV-transformed human B cells impose difficulties for further development into clinical use, as EBV is a relevant human pathogen. We have generated humanized mice expressing HLA-DR4 molecules in a NOD.RagKO.IL2RgcKO background (DRAG mice). Upon infusion of HLA-DR-matched human hematopoietic stem cells the DRAG mice develop functional human B cells able to mount IgM and IgG antibody responses to malaria parasites. Herein we show the generation of a panel of fully human mAbs to *Plasmodium falciparum* sporozoites and blood stage parasites that inhibit parasite development. The humanized DRAG mouse model thus represents an easy, convenient, and powerful approach for generation of fully human therapeutic or phrophylactic (i.e., transmission- blocking) mAbs.

1155

IMMUNOGENICITY AND TRANSMISSION BLOCKING ACTIVITY OF PFS25-BASED VACCINE CANDIDATES IN RHESUS MONKEYS

Yimin Wu[1], Craig Przysiecki[2], Jessica Chichester[3], Lynn Lambert[1], Sachy Orr-Ganzalez[1], Debbie Nahas[2], Erin Trexler-Green[2], Sarah Brockley[1], Olga Muratova[1], Joan Aebig[1], Xiaowei Wang[1], Charles Anderson[1], Dave Jones[1], David Narum[1], Vidadi Yusibov[3], Danilo R. Casimiro[2], Patrick E. Duffy[1]

[1]National Institutes of Health, Rockville, MD, United States, [2]Merck Research Laboratory, West Point, PA, United States, [3]Fraunhofer-CMB, Wilmington, DE, United States

The Pfs25 protein is a lead candidate for transmission blocking vaccine (TBV) against malaria. The main challenge for recombinant Pfs25-based vaccine development is to increase functional immunogenicity of Pfs25. Previous studies demonstrated that conjugating *Pichia*-produced Pfs25 to protein carriers such as Outer Membrane Protein Complex (OMPC) from *N. meningitides* or detoxified ExoProtein A (EPA) of *P. aeruginosa* resulted in higher functional immune responses in animals. A Pfs25-EPA conjugate vaccine is currently being evaluated for safety, immunogenicity, and transmission blocking activity in a Phase 1 trial. Recombinant Pfs25 has also been expressed in plant tissues. The lead product on clinical development path is Pfs25-VLP, plant-produced Pfs25-AIMV coat protein fusion products self-assembled as a viral-like particle. A Phase 1 study on

Pfs25-VLP/Alhydrogel is scheduled to begin in 4th quarter 2013. A rhesus study is in progress to evaluate functional immunogenicity of 3 Pfs25-based products, Pfs25-EPA, Pfs25-OMPC and Pfs25-VLP, all formulated with aluminum adjuvants. The animals received 3 immunizations on D0, D56, and D112 or D168, similar to the dose-regimen designs in human trials. This design would allow evaluation whether rhesus can be used in preclinical studies to predict responses in humans. Antibody responses are measured by Pfs25-specific ELISA. Transmission blocking activity of vaccine-induced antibodies is measured by standard membrane feeding assays. The animals are being followed up to 12 months post the 3rd vaccination, for evaluation of response duration. Results from this study may facilitate candidate selection process for TBV product development.

1156

PLASMODIUM VIVAX ANTIGEN DISCOVERY BASED ON ALPHA HELICAL COILED-COIL PROTEIN MOTIFS

Nora Cespedes[1], Angélica Castellanos[1], Mary Lopez-Pérez[1], Myriam Arévalo-Herrera[1], Ingrid Felger[2], Giampietro Corradin[3], Sócrates Herrera[4]

[1]Malaria Vaccine and Drug Development Center, Cali, Colombia, [2]Swiss Tropical and Public Health Institute, Basel, Switzerland, [3]Department of Biochemistry, University of Lausanne, Epalinges, Switzerland, [4]Caucaseco Scientific Research Centre, Cali, Colombia

Protein α-helical coiled-coil structures that elicit antibody responses able to block critical functions of medically important microorganisms represent a mean for vaccine development. By using bioinformatics algorithms of *Plasmodium falciparum* genome, functional α-helical coiled-coil structures were identified, chemical synthesis and multiple potential vaccine candidates are currently in pre-clinical and clinical development. We have used the same technology and approach to identify *P. vivax* antigens orthologous to *P. falciparum* antigens. A total of 40 proteins were evaluated for their antigenicity using sera samples of individuals from malaria endemic areas of Colombia; additionally, 8 of them were tested for their immunogenicity in BALB/c mice. Mouse antibody responses were determined by ELISA using as antigen the corresponding polypeptide and their reactivity with the native proteins was assessed by IFA test and western blot techniques using *P. vivax* blood stages. Analysis of these antigens reveals that such structures are highly antigenic and immunogenic, all fragments induced antibody response with titers variables from 104 to106 regarding to the antigen used. Additionally, the cellular response was evaluated through ELIspot assay for IFN-γ production in splenocytes from immunized mice. Six of the eight individuals induced IFN-γ production with media SFC from 60 to 300. Based on this results five protein fragments have been selected for immunogenicity and protective efficacy in Aotus monkeys. This approach combining α-helical coiled coil protein motifs and chemical synthesis could lead to the rapid identification and development of new malaria vaccine candidates.

1157

A CHIMERIC *PLASMODIUM FALCIPARUM* MEROZOITE SURFACE PROTEIN VACCINE INDUCES HIGH TITERS OF PARASITE GROWTH INHIBITORY ANTIBODIES

James R. Alaro[1], Andrea Partridge[2], Kazutoyo Miura[1], Ababacar Diouf[1], Ana M. Lopez[2], Evelina Angov[3], Carole A. Long[1], **James M. Burns, Jr.**[2]

[1]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [2]Drexel University College of Medicine, Philadelphia, PA, United States, [3]Walter Reed Army Institute of Research, Silver Spring, MD, United States

The C-terminal 19 kDa domain of *Plasmodium falciparum* merozoite surface protein 1 (*Pf*MSP1$_{19}$) is an established target of protective antibodies. However, clinical trials of *Pf*MSP1$_{42}$, a lead blood-stage vaccine candidate which contains the protective epitopes of *Pf*MSP1$_{19}$, revealed suboptimal immunogenicity and efficacy. Based on proof-of-concept studies in the *Plasmodium yoelii* murine model, we produced a chimeric vaccine antigen containing r*Pf*MSP1$_{19}$ fused to the N-terminus of *P. falciparum* merozoite surface protein 8 that lacked its low-complexity Asn/Asp-rich domain, r*Pf*MSP8 (ΔAsn/Asp). Immunization of mice with the chimeric r*Pf*MSP1/8 vaccine elicited strong T cell responses to conserved epitopes associated with the r*Pf*MSP8 (ΔAsn/Asp) fusion partner. While specific for *Pf*MSP8, this T cell response was adequate to provide help for the production of high titers of antibodies to both *Pf*MSP1$_{19}$ and r*Pf*MSP8 (ΔAsn/Asp) components. This occurred with formulations adjuvanted with either Quil A or with Montanide ISA 720 plus CpG ODN and was observed in both inbred and outbred strains of mice. *Pf*MSP1/8 induced antibodies were highly reactive with two major alleles of *Pf*MSP1$_{19}$ (FVO and 3D7). Of particular interest, immunization with *Pf*MSP1/8 elicited higher titers of *Pf*MSP1$_{19}$ specific antibodies when compared to a combined formulation of r*Pf*MSP1$_{42}$ and r*Pf*MSP8 (ΔAsn/Asp). As a measure of functionality, *Pf*MSP1/8-specific rabbit IgG was shown to potently inhibit the *in vitro* growth of blood-stage parasites of the FVO and 3D7 strains of *P. falciparum*. These data support the further testing and evaluation of this chimeric *Pf*MSP1/8 antigen as a component of a multivalent vaccine for *P. falciparum* malaria.

1158

CROSS-REACTIVITY OF ANTIBODIES FROM COLOMBIAN MEN AND CHILDREN EXPOSED TO *PLASMODIUM VIVAX* WITH THE *P. FALCIPARUM* VAR2CSA ANTIGEN

Sedami Gnidehou[1], Justin Doritchamou[2], Maria Isabel Arroyo[3], Eliana Arango[3], Ali Salanti[4], Nicaise Ndam[5], Amanda Maestre[3], Stephanie Yanow[6]

[1]University of Alberta, Edmonton, AB, Canada, [2]Université Paris Descartes, Paris, France, [3]Universidad de Antioquia, Medellin, Colombia, [4]University of Copenhagen, Copenhagen, Denmark, [5]Institut de Recherche pour le Développement, Paris, France, [6]ProvLab, University of Alberta, Edmonton, AB, Canada

During pregnancy, red blood cells infected with specific variants of *Plasmodium falciparum* sequester in the placenta through adhesion to chondroitin sulfate A (CSA). Sequestration is mediated by expression of PfEMP1 encoded by *var2csa*-type genes. Antibodies against these variants are associated with protection from maternal malaria. In most malaria-endemic settings, antibodies against VAR2CSA are infrequently observed in men, children, and non-pregnant women. Using enzyme-linked immunosorbent assay (ELISA), we detected antibodies against multiple constructs of VAR2CSA among Colombian men and children exposed only to *Plasmodium vivax*. We showed that DBL5e specific IgGs were present at significant levels among men (54%) and children (70%), as well as antibodies to DBL3X and ID1-ID2. Moreover, results showed that Colombian men and children had anti-VAR2CSA antibodies with high avidity, and a predominance of IgG1 and IgG3 subclasses. By competition ELISA assays, we demonstrated that purified IgG from men and naturally antibodies acquired by immune Beninese multigravidae shared common epitopes with VAR2CSA antibodies induced in immunized rabbits. These findings suggest that exposure to *P. vivax* can generate antibodies against VAR2CSA from *P. falciparum* in the general population in Colombia, which may alter the pathogenesis of malaria in regions where both species co-exist.

1159

DRAG MICE WITH A HUMAN-IMMUNE SYSTEM, DEVELOP HUMAN HEPATOCYTES AND ERYTHROCYTES AND SUSTAIN THE COMPLETE LIFE CYCLE OF *PLASMODIUM FALCIPARUM* MALARIA PARASITE

Wathsala Wijayalath[1], Sai Majji[1], Yulia Kleschenko[1], Megan Dowler[2], Andrea Akyeampong[1], Eileen Villasante[1], Thomas L. Richie[1], Teodor-Doru Brumeanu[3], Sofia Casares[1]

[1]U.S. Military Malaria Vaccine Program, Naval Medical Research Center/ Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Entomology Branch, Walter Reed Army Institute of Research, Silver Spring, MD, United States, [3]Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD, United States

Malaria is a deadly infectious disease affecting millions of people in the tropical countries across the world. Among the five species of *Plasmodium* parasites that affect humans, *P. falciparum* accounts for most morbidity and mortality associated to malaria. The lack of convenient animal models able to sustain liver- and blood-stage *P. falciparum* infection has hindered the understanding of disease pathogenesis and vaccine testing prior to clinical trials. We have previously showed that humanized DRAG (HLA-DR4.RagKO.IL2RgcKO.NOD) mice infused with hematopoietic stem cells from umbilical cord blood generate a functional human immune system and respond to vaccination. Herein, we show that the humanized DRAG mice also develop human hepatocytes and human erythrocytes and sustain blood-stage *parasitemia* upon challenge with *P. falciparum* sporozoites. DRAG mice thus represent the first small rodent model to support the complete life cycle of *P. falciparum*. The ability of DRAG mice to develop a functional human immune system and sustain *P. falciparum* infection provides a unique model to test the immunogenicity and protective efficacy of human malaria vaccine candidates.

1160

CSP AND AMA-1 ANTIGEN-SPECIFIC REFERENCE REAGENTS FOR THE STANDARDIZATION OF MALARIA VACCINE CELLULAR IMMUNOLOGIC ASSAYS

Alexandra L. Singer, Martha Sedegah, Cindy Tamminga, Bradley Hickey, Thomas Richie, Eileen Villasante, Judith Epstein

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

Malaria causes significant mortality worldwide, and there is an urgent public health need for a vaccine. Several promising candidate vaccines to prevent *Plasmodium falciparum* are currently being evaluated in clinical trials around the world. Of these, the best vaccines will be down-selected based on favorable safety and tolerability profiles, superior immunogenicity and high protective efficacy. The down-selection process with regard to immunogenicity would be improved by the generation of standard reagents that could then be used as negative and positive controls across diverse assays for cell mediated immunity (CMI). Several barriers have existed to generating such reagents: peripheral blood mononuclear cells (PBMCs) from human research subjects are limited in quantity, antigen-specific cellular responses have generally been low, and until recently, there have been no candidate malaria vaccines that predictably induced strong CD8+ T cell responses in humans. The Malaria Department of the Naval Medical Research Center now has an opportunity to generate these reagents using the NMRC-M3V-AdPfCA Vaccine, a highly immunogenic adenovirus (serotype 5)-vectored vaccine encoding the Pf CSP (expressed in sporozoite and early liver stages) and PfAMA1 (expressed in sprozoite, liver and erythrocytic stages) antigens. In our experience, a single, low dose immunization with this vaccine (2x1010 particle units), delivered intramuscularly without adjuvant, induces brisk antigen-specific cellular immune responses (average IFN-γ secreting cells to CSP of 400-500 per million PBMC and to AMA1 of 900-1100 per million PBMC). For this reason, we are conducting a Phase 1 trial in which eligible subjects will receive a single administration of the Ad-PfCA vaccine with large quantities of PBMCs collected via leukapheresis pre- and post-immunization. We anticipate that this clinical trial will provide invaluable reagents to expedite the development and optimization of novel cell-mediated assays.

1161

A RETROSPECTIVE ANALYSIS OF THE ADVERSE EVENT DATA FROM THE PHASE 1 TRIAL *PLASMODIUM SP.* SPOROZOITES IMMUNIZATION OF HUMAN VOLUNTEERS

Bradley W. Hickey, Joanne Lumsden, Sharina Reyes, Yara Elbeshbishi, Andrew Mix, Jade Spurgeon, Thomas Luke, Daniel Freilich, Eileen Villasante, Judith Epstein, Thomas Richie

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

In 2002-2003, we immunized research subjects with radiation-attenuated *Plasmodium falciparum* sporozoites (RAS) via mosquito bite, a model for vaccination against malaria that historically has induced high grade (>90%) protection against controlled human malaria infection (CHMI) following > 1000 immunizing bites. Leukapheresis was conducted before and after RAS immunization to collect large numbers of peripheral blood mononuclear cells (PBMC) to characterize protective immune responses and identify protective antigens for malaria vaccine development. Volunteers were placed in two main groups: immunization with RAS, and mock immunization with uninfected mosquitoes. We herein report the safety data collected during the trial. 57 volunteers were screened, 41 enrolled, and 27 received at least one immunization session. Of the 27, source documentation for 22 was available for retrospective analysis. The results revealed that immunization via mosquito bite was generally well tolerated, but could also lead to significant adverse events (AEs). Local AEs were consistent with reactions to mosquito bites in the wild, consisting of erythema, papules, swelling, and induration; however, two individuals, one true and one mock immunized, developed generalized swelling of the forearm (large local reactions) and were withdrawn from further participation. Systemic AEs were generally rare and mild, consisting of headache, myalgias, nausea, and low grade fever; however, two subjects experienced the abrupt onset of a symptom complex characterized by fever, malaise, myalgia, nausea and rigors consistent with a form of serum sickness secondary to a concentration of pre-formed antibody reacting with mosquito salivary antigens. Overall, our retrospective analysis of *Pf*RAS immunization via mosquito bite revealed that, although the method is generally safe and well tolerated, large local reactions to the mosquito bites and systemic adverse reactions of moderate severity may occur, and may relate more to mosquito salivary antigens than to the malaria sporozoites.

1162

PLASMODIUM VIVAX SPOROZOITE CHALLENGE METHOD FOR MALARIA VACCINE AND DRUG CLINICAL DEVELOPMENT

Myriam Arévalo-Herrera, David A. Forero, Kelly Rubiano-Cardona, **Julian E. Muñoz**, Mary Lopez-Perez, Sócrates Herrera

Caucaseco Scientific Research Center, Cali, Colombia

Important efforts have been performed towards the development of malaria vaccines including *Plasmodium vivax*. We have established a safe, reliable, and reproducible sporozoite infectious challenge method in Colombia. Two infection trials have been conducted in naïve human volunteers that have been exposed, in a first challenge to the bite to 2-4, 5-7 and 8-10 mosquitoes of *Anopheles albimanus* mosquitoes infected from one *P. vivax* donor and in a second challenge, volunteers where exposed to the bites of 2-4 mosquitoes infected from different donors. Pre-patent periods were similar in both trials, between 9-13 days (mean 10.6) in the first and 9-16 days (mean 12) in the second trial as determined by Thick Blood Smear. All volunteers were closely followed for clinical appearance of malaria and where treated immediately *parasitemia* became patent. All participants successfully recovered from malaria after treatment, with no serious adverse events. In preparation for Phase IIa/

IIb vaccine trials, a third trial has been designed to determine differences between naïve individuals and volunteers having been previously in contact with malaria (pre-immune). We will compare the length of pre-patent period, clinical manifestations and immune responses of both groups. A total of 19 volunteers, 7 naïve from a non-endemic area and 12 pre-immune from endemic area were enrolled. Volunteers are men (n=12) and female (n=7) with 18-45 y/age. Naïve volunteers have been confirmed to be negative for malaria by ELISA and IFAT, whereas pre-immune have displayed reactivity against PvMSP-1 and PvCS (1:200 titers both) by ELISA and *P. vivax* blood stages by IFAT (1:40 to 1:160 titers). Sera and cells of all volunteers will be collected before and during the trial to determine antibody and cell mediated responses. We would assess the induction of antibody responses in naïve volunteers and the potential boosting effect of infection in pre-immune volunteers. Additionally, cytokine levels, as well as both B cell and monocyte subpopulation profiles will be evaluated. Results of this ongoing trial will be presented.The described method is critical for phase IIa/b vaccine trials and is contributing to accelerate the clinical development of *P. vivax* vaccines and would be valuable for testing of antimalarial drugs targeting liver parasite forms.

1163

URINARY TRACT INFECTION: PREVALENCE, PATHOGENS AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN AMONG FEBRILE CHILDREN AT MWANANYAMALA HOSPITAL, TANZANIA

Aileen K. Barongo

Hubert Kairuki Memorial Universty, Dar es salaam, United Republic of Tanzania

Urinary tract infection (UTI) is a common and important cause of morbidity in the pediatric population in developing countries. Prevalence rates of UTI in children ranges from 3.3% in the United States to 39.7% in Northwestern Tanzania.Many uropathogens are developing resistance to antibiotics recommended by WHO to treat UTI.The magnitude, etiology and antimicrobial susceptibility of UTI in Tanzanian febrile children are not well defined. All published studies to date on the prevalence and etiologies of UTI in febrile children have not included a control group which is necessary to investigate false positives due to poor collection procedures. The aim of the study was to determine the prevalence of UTI, pathogens and antimicrobial susceptibility in febrile children aged 2-5 years at Mwananyamala District Hospital (MDH). This was a cross-sectional descriptive hospital-based study. Febrile and afebrile children were consecutively recruited from the paediatric outpatient clinics at MDH. Urine culture and sensitivity testing was performed for febrile and afebrile children who had positive urine dipstick tests for nitrates and leukocyte esterase. A total of 556 children were enrolled into the study.Of these 370(66.5%) were febrile and 186(33.5%) were afebrile.Prevalence of UTI among febrile children was 7.8%. Females had higher prevalence than males, however, the difference was not statistically significant(p=0.418). Prevalence of UTI among afebrile children was 5.9%. Febrile children were noted to have higher prevalence of UTI than afebrile children, though the difference was not statistically significant (p=0.729). *Escherichia coli* (38%) was the most commonly isolated organism followed by *Klebsiella* (21%). Resistance rates of the isolated bacteria to Cotrimoxazole, Erythromycin, and Amoxillin were 100%,89.7% and 86.2% respectively. Gentamycin and Ceftriaxone had slightly lower resistance rates of 48% and 57% respectively while Amikacin and Ciprofloxacin had least resistance rates of 0% and 6.9%respectively. In conclusion prevalence of UTI among febrile children aged 2-5 is 7.8% and the commonest bacteria isolated were *E. coli* and *Klebsiella* which showed a high resistance to Amoxillin, Cotrimoxazole and Erythromycin. Further long term studies should be conducted among this age group so as make changes to the current standard treatment for UTI.

1164

STUDIES OF *ORIENTIA TSUTSUGAMUSHI* PERSISTENCE IN NEWLY DEVELOPED ANIMAL MODELS

Guang Xu, Thomas R. Shelite, Nicole L. Mendell, Yenny Goez-Rivillas, Bin Gong, Lynn Soon, Juan P. Olano, Gustavo A. Valbuena, David H. Walker, Donald H. Bouyer

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

Orientia tsutsugamushi is a gram-negative obligately intracellular coccobacillus. It is the causative agent of scrub typhus, a serious public health problem in Asia and the islands of the Pacific and Indian oceans. It can cause severe multiorgan failure with a mortality rate of 7-15% without appropriate treatment. The antigenic heterogeneity of *Orientia* plays a role in scrub typhus reinfection. As a neglected disease, there are still gaps in understanding how *O. tsutsugamushi* invades, disseminates, and interacts within the host. We have developed new mouse models for *Orientia* infection, which better mimic the pathology and immunology in human patients. The histopathology and cytokines from relevant organs such as lung and liver confirmed that our intravenous (i.v.) and intradermal (i.d.) mouse models are valid for the study of scrub typhus. Real-time PCR and immunofluorescence staining demonstrated that kidney was the main target organ with 34,250 to 270, 000 copies of *O. tsutsugamushi* per kidney during the persistent infection after i.v. and i.d. inoculation. Seventy five percent fewer copies of *O. tsutsugamushi* were recovered from the corresponding lung samples, the main target during acute *Orientia* infection, than kidney. These bacteria persistent in the mammalian host were viable and virulent. Histologic studies showed lesions (vasculitis, and interstitial nephritis) in the renal tissue. We also immunosuppressed *Orientia* infected mice with cyclophosphamide or radiation 82 days post infection. The immunosuppressive treatments did not result in clinical recrudescence of scrub typhus. All kidneys, lungs, spleens, livers and lymph nodes collected from those immunosuppressed and infected mice contained virulent *O. tsutsugamushi* that were infectious for naive mice. We also found changes of host immune cells and their cytokines, but further studies are required. We are currently investigating the mechanisms behind these phenomena, and we hypothesize that *Orientia* finds and induces loopholes in host immunity to avoid being cleared.

1165

LEPROSY ELIMINATION IN BANGLADESH: A NEGLECTED DISEASE REVISITED IN 2012

Shaikh A. Shahed Hossain[1], Safir Uddin Ahmed[2], Mohhamad Hadi[3], Jalal Uddin Ahmed[2], Mannan Bangali[3], Aprue Mong[4], Md. Ashaque Husain[2]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [2]NLEP, Dhaka, Bangladesh, [3]World Health Organization Bangladesh, Dhaka, Bangladesh, [4]LEPRA, Dhaka, Bangladesh

In 2013, Leprosy remained as a neglected disease in Bangladesh. Though the WHO declared elimination goal was achieved in 1998, yet the national Leprosy elimination Programme (NLEP) statistics started to show stagnancy in prevalence, new case detection and variations in other parameters. This is more noticed in the north and north eastern part of the country, the area chronically suffers from poverty, seasonal famine and lack of common nutrients. Data from the national programme (NLEP) and its implementing partners are analyzed. Two workshops aiming at formulating elimination strategies for 2011-16 and 2015 were organized in 2012 and 2013. Issues and concerns from those meetings are incorporated. NLEP is detecting around 4-5 thousands new cases each year since 2006. However, out of 64 districts, 13 endemic areas having 25 % of the total population, contributing to 59% of the all the cases detected in 2012. Fifty percent of them were MB cases, 57% of the smear positive, 68% of the ≤ 15 years cases, and 58% of the visible disabilities cases belonged to these 13 areas. Loss of focus in elimination activities, less fund allocation, rapid burn out of skilled personnel at field level, and huge lack of awareness about the

disease are major concerns. Complacency in elimination activities and lack of any interest in leprosy elimination created an emergent threat of this neglected disease affecting mostly the poor people of the society.

1166

MODULATION OF IMMUNIZATION VIA CHOLINERGIC NERVOUS SYSTEM USING HI-6

Miroslav Pohanka

Faculty of Military Health Sciences, University of Defense, Hradec Kralove, Czech Republic

HI-6 is a compound known is some sources as asoxime. It is used as an antidote to nerve agents. When HI-6 administered to the poisoned one, it causes return of acetylcholinesterase (AChE) activity. In recent works, we proved that HI-6 acts as an antagonist to acetylcholine receptors (AChR) including the nicotinic receptor, alpha 7 nAChR which is involved in regulating the immune response through macrophages. The here presented experiment reports the efficacy of HI-6 to regulate the immune response. Laboratory BALB/c mice received HI-6 and/or keyhole limpet hemocyanin (KLH) as an antigen. Controls received saline or a combination of Freund´s complete adjuvant and KLH. Antibody production was investigated after either 21 or 65 days when either single or repeated dose of antigen was applied. We confirmed that HI-6 significantly improved vaccination efficacy when KLH was given in a dose of 1 mg/kg. The effect was dose dependent: repeated HI-6 produced no on further improvement of the vaccination. A combination of HI-6 and KLH produced a vaccination of almost the same efficacy as that for Freund´s complete adjuvant. The findings point at the suitability of HI-6 for improving vaccination efficacy at the level of immunity regulation by the nervous system.

1167

STAPHYLOCOCCUS AUREUS ADHESION-GENES EXPRESSION IN AN ORAL EPITHELIUM IN VITRO MODEL

Gloria Paniagua, Eric Monroy, Sergio Vaca

Universidad Nacional Autónoma de México, FES Iztacala, Tlalnepantla, Edo. de México, Mexico

Staphylococcus aureus is an important nosocomial pathogen able to produce a great number of extracellular virulence factors and molecules associated to the cell wall, including several adhesins, such as the microbial surface components recognizing adhesive matrix molecules (MSCRAMMs). The aim of this work was to determine the expression of 12 adhesins in *Staphylococcus aureus* strains, isolated from catheters of hemodialysis patients, in an oral epithelium *in vitro* model. *S. aureus* was identified by manitol and coagulase biochemical tests, and by rRNA, *nuc* and *spa* (x region) PCR amplification. *S. aureus* strains were also investigated by PFGE (pulsed field gel electrophoresis) by *SmaI* DNA digestion and electrophoresis in CHEF MAPPER (Bio-Rad). After *S. aureus* oral epithelium infection, *S. aureus* RNA was extracted and subjected to reverse transcription and real time PCR of adhesion genes using Qiagen comercial kits. All *S. aureus* strains (n=21) expressed *bbp, clfb*, *cna,* and *sdrC* genes*;* 95.2% (n=20) *sdrD;* 90.4% (n=19) *map/eap;* 85.7% (n=18) *ebps*; 76.1% (n=16) *sdrE;* 47.6 (n=10) *fnbA, fnbB,* and *spa;* 28.5% (n=6) *clfA.* Results showed that *S. aureus strains* expressed different combinations of MSCRAMMs´s family genes during oral epithelium infection. Analysis of DNA restriction fragments by PFGE revealed that all *S. aureus* strains are different, suggesting that catheter contamination is not due to the hospital personnel manipulating hemodialysis equipment.

1168

DIFFERENTIAL GROWTH OF *RICKETTSIA FELIS* STRAINS RF2125 AND URRWXCAL2 IN TWO CELL LINES

Laya Hun, Lizeth Taylor, **Adriana Troyo**

Universidad de Costa Rica, San Jose, Costa Rica

The importance of *Rickettsia* has increased in the past couple of decadesa, especially as intracellular bacteria responsible for emerging zoonotic diseases. *Rickettsia* felis is considered one of these emerging pathogens in Latin America, and it has been associated with human disease in Brazil, Mexico, and Colombia. Recently in Costa Rica, two different strains of *Rickettsia* felis from fleas have been isolated in cell culture. The aim of this study was to determine the growth characteristics of strains RF2125 and URRWXCal2 in Vero and C6/36 cell lines. Two isolates of R. felis strain RF2125 and one of strain URRWXCal2 all from Ctenocephalides felis of Costa Rica were inoculated at 1.38x106 bacteria/ml in bottles with confluent monolayers of C6/36 cells in RPMI with 2.5% fetal calf serum and Vero cells in MEM with 4% newborn calf serum. Growth was evaluated in both cell lines with and without a 2% triptose supplement and at 28 °C and 32 °C. Bacterial growth was evaluated every week for a period of one month, using a semiquantitative scale of 1+ to 4+ (+ to ++++) according to the number of bacteria per cell observed with a Giménez stain. Both isolates of R. felis RF2125 grew well (+++ to ++++) in Vero cells with and without triptose at both temperatures, although growth was slower in one of the bottles without triptose at 32 °C. Growth of R. felis RF2125 isolates was minimal (+) after 4 weeks in C6/36 cells in medium with and without triptose and at both temperatures. *Rickettsia* felis isolate URRWXCal2 grew moderately (+++) in C6/36 cells at a 28 °C with and without triptose, as well as at 32 °C with triptose. Its growth was reduced (++) at 32 °C without triptose, and it only slightly grew (+) in Vero cells at 28 °C with triptose. Both isolates of R. felis RF2125 exhibited similar growing characteristics, with better growth in Vero cells. The isolate of R. felis URRWXCal2 was completely different as it grew better in C6/36 cells than in Vero cells. A triptose supplement in the medium favored growth of both R. felis strains in cell culture. Results show that there are differences at the metabolic level and/or specific receptors that should be further evaluated.

1169

ASSESSMENT AND COMPARISON OF THE HUMAN IMMUNOLOGICAL RESPONSE TO ANTHRAX INFECTION AND VACCINES

Wendy M. Webster[1], Tinatin Kuchuloria[2], Amanda K. Debes[1], Stephen F. Little[1], Shota Tsanava[3], Nazibrola Chitadze[3], Salome Saghinadze[3], Nikoloz Tsertsvadze[3], Paata Imnadze[3], Thomas R. Laws[4], Hugh E. Dyson[4], Andrew J. Simpson[4], Robert G. Rivard[1], Mathew J. Hepburn[5], **Nino Trapaidze**[2]

[1]U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, United States, [2]Walter Reed Army Institute of Research-United States Army Medical Research Institute for Infectious Diseases Clinical Research Unit, Walter Reed Army Institute of Research Georgia Project, Tbilisi, Georgia, [3]National Center for Disease Control and Public Health, Tbilisi, Georgia, [4]Defense Science and Technology Laboratory (DSTL), Porton Down, United Kingdom, [5]U.S. Army Medical Command (Majority of contribution occured while Dr. Hepburn was assigned to United States Army Medical Research Institute for Infectious Diseases), San Antonio, TX, United States

Understanding immune responses to anthrax remains a priority in biodefense countermeasure development. Currently, three different anthrax vaccines are employed for human protection. The US Anthrax Vaccine Adsorbed (AVA, BioThrax) represents a cell free preparation of protective antigen (PA). Live attenuated anthrax vaccine (LAAV) is licensed for human use in former Soviet Union countries. Wecompared immune responses elicited by different vaccines with those in patients diagnosed with cutaneous anthrax.Sixty three clinical serum samples were evaluated

from subjects with either LAAV (n=16) or AVA (n=11) vaccinations, cutaneous anthrax patients (n=28) and naïve controls (n=8). Samples were tested in quantitative anti-PA, endpoint anti-LF and anti-EF IgG ELISAs, and toxin neutralization assay (TNA). Antibody titer data was transformed to remove unequal variance and analyzed by multivariate linear modelling. Significantly raised anti-PA antibody titers, when compared to controls (geometric mean (GM) = 0.23 µg/mL), were found in the anthrax patients (GM = 3.14 µg/mL, P=0.001) and the AVA recipients (GM = 35.63 µg/mL, P<0.001) and weakly higher in LAAV vaccinees (GM = 1.78 µg/mL, P=0.096). Strongest anti PA antibody responses were observed in the AVA vaccinees with levels significantly higher than those in other groups (P<0.002,). Anthrax patients demonstrated significantly raised anti-LF responses (P<0.001) when compared to controls. No significantly raised anti-EF responses were observed (P>0.10). The TNA ED50 titers were compared by Dunn's multiple comparisons. We found that TNA ED50 titers were significantly raised, when compared to controls (0% responding), in the AVA (72.7% responding, P=0.012) vaccinated group, but not the anthrax patients (39.3% responding, P=1.0) or the LAAV vaccinees (18.8% responding, P=1.0). We found that none of the factors, time elapsed since exposure anthrax antigen, gender and subject age effected antibody titer with the exception of number of doses effecting anti PA antibody titres (P=0.001), used as a covariate in multivariate analysis of antibody data. The vaccines included in this study are regarded as protective and the variations in human response to these vaccines observed here are a demonstration that correlates of protection need to be thought out carefully.

1170

LEPROSY AMONG THE FOREIGN-BORN IN THE UNITED STATES, 2000-2010

Stephen Waterman[1], Alfonso Rodriguez[1], Richard Truman[2], David Blaney[3]

[1]Centers for Disease Control and Prevention, San Diego, CA, United States, [2]Louisiana State University School of Veterinary Medicine, Baton Rouge, LA, United States, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States

Leprosy (Hansen's disease, HD) is a neglected tropical disease and, although rare, may cause severe disability. Over the last 30 years, the HD burden has declined worldwide, in large part due to implementation of free short-course multiple-drug therapy. Still, HD remains endemic in many less-developed countries. In the United States (US), HD is a rare disease mostly associated with immigrants from endemic areas. We reviewed surveillance data on HD in the US, with a focus on foreign-born populations. Data were obtained from the National Hansen's Disease Registry for the period 2000-2010. We used chi-square and t-tests to compare characteristics of foreign-born and US-born cases. A total of 1,439 HD cases were diagnosed during 2000-2010, an average of 130 cases per year. Persons with cases resided in 49 states, the top five states being Texas (15%), California (14%), Hawaii (10%), Florida (8%), and Louisiana (7%). Seventy-three percent of cases were in foreign-born persons, for whom the top countries of birth were Mexico (20%), Micronesia and Trust Territories (18%), Brazil (12%), the Philippines (11%), and India (11%). Rates of HD among the foreign-born peaked in 2003 (0.33 per 100,000) and declined to 0.21 per 100,000 by 2010. Rates for the US-born remained stable (0.01 to 0.02 per 100,000) during the study period; in 2010 rates among the foreign-born were 21 times higher than among the US-born. Seventy percent of cases were in males. The median age at diagnosis was significantly lower (p<0.05) for the foreign-born (35 years) than the US-born (56 years). The median time from arrival in the US to diagnosis was 6.3 years. Leprosy remains a health disparity for the foreign-born in the US. Persons from endemic countries need to be targeted for disease education, case-finding, and case-management interventions. Further research is needed on the epidemiology of HD in the US, and specifically on the reasons for diagnosis of HD years after arrival in the US.

1171

AR-12: A BROAD SPECTRUM HOST CELL-DIRECTED INHIBITOR OF INTRACELLULAR PATHOGENS

Hassan Borteh, Ky V. Hoang, Murugesan V. Rajaram, Heather Curry, Eric M. Bachelder, John S. Gunn, Larry S. Schlesinger, **Kristy M. Ainslie**

The Ohio State University, Columbus, OH, United States

AR-12 is an IND approved, COX-2 inhibitor derivative that affects host cells primarily by up-regulating autophagy. In vitro, AR-12 has shown broad-spectrum efficacy against several bacteria and parasites including *Salmonella typhimurium* and *Francisella tularensis*. Although for some pathogens, the drug's effect is observed directly against the pathogen, AR-12 shows its strongest promise as a host-targeted therapeutic, which makes it ideal for treatment of multidrug resistant agents. In vitro analysis of AR-12 for *S. typhimurium* -infected macrophages displayed a MIC of 0.5 µM; however, 5 mg AR-12/kg in BALB/c mice did not result in significant increase in overall mouse survival with S. Typhimuriumm infection. Reduced *in vivo* efficacy is likely due to drug dosing limits because of poor drug solubility and biodistribution. To overcome solubility issues, we have encapsulated AR-12 in acetalated dextran (Ac-DEX) nanoparticles (NPs) that passively target phagocytes (the preferred host cell of these bacteria), while non-phagocytic cells are not capable of internalizing the particles. By passively targeting the phagocytes, we can achieve targeted delivery of the compound directly inside these cells, thus limiting drug side effects and perhaps enhancing the action of the drug, compared to unencapsulated parenteral administration. Ac-DEX is an acid sensitive polymer with tunable release kinetics that will degrade and release drug in the low pH environment of phagocytes' phagosome. This biodegradable polymer is superior to other commonly used biomaterials (e.g. poly lactic-co-glycoic acid (PLGA)) because it degrades into pH neutral dextran and low levels of ethanol and acetone. We have demonstrated that AR-12 encapsulation into Ac-DEX NPs can significantly reduce drug-induced cytotoxicity in human monocyte-derived macrophages (MDMs) and that higher drug concentrations can be achieved intracellularly. Furthermore, we have evaluated the encapsulated drug against *Mycobacterium tuberculosis*, *S. typhimurium* and *F. tularensis* in host MDMs. These studies will help to develop and characterize a new broad-spectrum antibiotic and delivery platform that targets the host and inhibits bacterial survival by increased co-localization of intracellular bacteria with autophagosomes. Such a platform would help eliminate the pathogen and limit the emergence of multidrug resistance.

1172

FREQUENCY OF GES β-LACTAMASES IN *PSEUDOMONAS AERUGINOSA* CLINICAL ISOLATES *IN* LIMA, PERÚ

Paul Rios, Rina Meza, **Mark P. Simons**, Drake Hamilton Tilley

U.S. Naval Medical Research Unit - 6, Callao, Peru

GES β-lactamases are a group of enzymes with both extended-spectrum beta-lactamase (ESBL) and weak carbapenemase hydrolysis activity. The GES β-lactamases play an important role in the spread of antibiotic resistance and complicate treatment choices for *Pseudomonas aeruginosa* infections. GES β-lactamases in multidrug resistant *P. aeruginosa* have been widely described worldwide, however scarce information is available in Latin American countries. We tested 149 *P. aeruginosa* clinical isolates obtained in 5 hospitals from Lima during July 2010 to July 2012. Resistance patterns were determined by the disk diffusion test and interpreted according to CLSI guidelines. Multidrug resistant was defined as the resistance to at least two of the following antibiotics families: β-lactams, quinolones, or aminoglycosides. To screen for GES β-lactamase activity, the double disk diffusion phenotypic test using ceftazidime and imipenem disks was performed and the genes encoding VEB, PER, TEM, SHV and OXA-1-like β-lactamases were detected by PCR. We additionally performed PCR for the detection of GES β-lactamases genes and determined the total frequency of GES β-lactamases among the *P.*

aeruginosa isolates to be 42% (63/149) with a 7% (10/149) concordance with the isolates phenotype. The frequency of genes encoding TEM and OXA-1-like β-lactamases was 7% and 13%, respectively. Genes encoding VEB, PER and SHV were not detected in these clinical isolates. Among the GES β-lactamases producers, we found that 13% (8/63) of these isolates also had genes encoding TEM β-lactamases and 8% (5/63) had genes encoding OXA-1-like β-lactamases. In addition, 87% of the *P. aeruginosa* isolates were multidrug resistant. There was a strong association between the presence of GES β-lactamases genes and those isolates which were multidrug resistant ($p<0.001$). In conclusion, 35% of the GES-positive *P. aeruginosa* isolates would have been missed by conventional phenotypic methods and supports the use of molecular methods to more reliably detect antibiotic resistance within the hospital.

1173

POINT-OF-CARE DIAGNOSIS FOR THE EMERGING SKIN DISEASE, *BURULI* ULCER: AN ANTIGEN CAPTURE APPROACH

Katharina Röltgen[1], Anita Dreyer[2], Jean-Pierre Dangy[1], Martin W. Bratschi[1], Marie-Thérèse Ruf[1], Sarah Kerber[1], Dorothy Yeboah-Manu[3], Gerd Pluschke[1]

[1]Swiss Tropical and Public Health Institute, Basel, Switzerland, [2]Seattle Biomedical Research Institute, Seattle, WA, United States, [3]Noguchi Memorial Institute for Medical Research, Legon, Ghana

Buruli ulcer (BU) is a chronic and destructive skin disease caused by infection with *Mycobacterium ulcerans*. Since the mode of transmission is still not entirely explored and there is no vaccine to prevent the disease, the current strategy to control BU relies on early case detection and antibiotic treatment. In remote areas of West and Central Africa, which are most affected by the disease, the diagnosis of BU is primarily based on clinical findings. However, the differential diagnosis for non-specific early stages of BU is broad. A specific and sensitive diagnostic test, which can be performed without expensive equipment and qualified personnel, could greatly support the diagnosis of M. ulcerans infection at field sites. While we have shown in our previous work that antigenic cross-reactivity between different mycobacterial species complicates the development of a specific serological test, we are now focusing on a direct detection of M. ulcerans antigens within the BU lesions. For this purpose, we generated high affinity antibodies against a set of highly abundant M. ulcerans protein antigens and evaluated their suitability as key reagents in ELISA-based antigen capture assays. Analyses of partial swab samples taken from BU lesions revealed for one of the antigens a sensitivity rate comparable to that of microscopic detection of acid fast bacilli in smears from clinical specimens. Systematic optimization of ELISA parameters further improved the sensitivity of antigen detection. We are now testing full BU swab samples in order to compare the performance of the developed assay with real-time PCR analysis, which is the current gold standard for BU diagnosis in reference laboratories. In addition, we are analyzing the potential application of the developed immunological reagents in other field-compatible assay formats.

1174

DETECTION OF MUTATIONS IN GYRA GENE OF FLUOROQUINOLONE-RESISTANT *NEISSERIA GONORRHOEAE FROM* SWABS AND URINE SAMPLES

Liz Sanchez[1], Nilda Gadea[1], Jesse Clark[2], Eddy Segura[2], Javier Lama[3], **Mark P. Simons**[1], Drake Hamilton Tilley[1], Jorge Sanchez[3], Silvia M. Montano[1]

[1]U.S. Naval Medical Research Unit - 6, Callao, Peru, [2]University of California Los Angeles, Los Angeles, CA, United States, [3]Asociacion Civil Impacta Salud y Educacion, Lima, Peru

Gonorrhea is the second most commonly reported bacterial sexually transmitted infections (STI), and a public health problem of great importance. Approximately 95% of heterosexual men are symptomatic, while more than 60% of women may be asymptomatic carriers. Among men who have sex with men (MSM), pharyngeal and rectal infections are common and mostly asymptomatic. Increasing antibiotic resistance presents a significant problem for gonorrhea treatment and it is essential to maintain continuous monitoring of strains for resistance to first-line antibiotics such as fluoroquinolones. In this study 1140 samples (pharyngeal and rectal swabs and urine) were tested by the GenProbe APTIMA CT/GC Transcription Mediated Amplification (TMA) assay which yielded 59 *Neisseria gonorrhoeae* positive-samples. Of these 11 of the 59 (18.6%) samples were found resistant to fluoroquinolones (QRNG) by Real Time PCR. Four (6.8%) were heterozygous, 14 (23.7%) had wild-type *N. gonorrhoeae* and 34 (57.6%) did not amplify. QRNG were sequenced and analyzed and identified one (9.1%) with single mutation S91I, two (18.1%) with single mutation S91F, two (18.1%) with double mutations S91F and D95A and six (54.6%) with double mutations S91F and D95G in the gyrA gene.In conclusion, detecting QRNG from invasive and noninvasive samples is important for monitoring antimicrobial resistance. In the last decade, fluoroquinolones have been used as antibiotics to treat gonorrhea but in recent years reports of quinolone-resistant strains have appeared in several countries. The presence of QRNG with double mutation (S91 and D95) indicates a high resistance to quinolones and suggests they should no longer be used as first-line therapy in Peru.

1175

IMPLEMENTATION OF AUTHORITY GUIDELINES AND INNOVATION WITH EMPHASIS ON PREVENTION OF HEALTHCARE-ASSOCIATED SYMPTOMATIC CATHETER-ASSOCIATED URINARY CATHETER INFECTIONS (CAUTIS)

Abimbola Ogundimu

University of Georgia, Alpharetta, GA, United States

The 600-bed hospital embraced the Centers for Medicare and Medicaid Services (CMS) challenge to address healthcare-associated infections (HAIs) as "never-events" by focusing on improving team communication, patient safety and quality through interventions to decrease the rates of symptomatic healthcare catheter-associated urinary catheter infections (CAUTIs). The Hospital Board of Directors established a goal to reduce the count of symptomatic catheter-associated UTIs by 10%, from a baseline (Jan 2008-Dec 2008) of 57 symptomatic CAUTIs to 51 symptomatic CAUTIs in Fiscal Year/FY 2010 (July 2009-June 2010). A multidisciplinary project management team, directed by the Infection Prevention and Control (IPC) team, included Clinical Nurse Specialists/CNS; front line staff; the IPC Committee; Clinical Analysts; Medical Staff; Chief Medical Officer; and Clinical Educators) used to implement the initiative. A urinary catheter bundle was implemented by: setting clear expectations for indications for indwelling urinary catheter use (an indwelling catheter policy which included criteria for catheter use); catheter insertion using aseptic technique and sterile equipment; use of small size catheters; catheter maintenance; hand hygiene; documentation of catheter placement); daily assessment of the continued need for indwelling urinary catheter by a multidisciplinary team (bedside Nurses, CNS, IPC and Case Management); the use of a bladder scanner before catheter placement; the use of intermittent catheterization when indicated and the promotion of education of direct care givers in clinical areas when CAUTIs occurred. The hospital realized a 63% reduction in symptomatic CAUTIs from MRSA cases of colonization and infection between the 2008 period (Jan 1, 2008-Dec 31, 2008) and 2009 period (Jan 1, 2009-Dec 31, 2009). The fiscal year-to-date (through March 2010) rate was 17 cases of CAUTIs, which was well below the target of 51 cases for the FY (which ended in June 30, 2010).

1176

PATHOGENESIS AND TREATMENT OF *BURULI* ULCER - A HISTOPATHOLOGICAL PERSPECTIVE

Gerd Pluschke, Marie-Thérèse Ruf

Swiss Tropical and Public Health Institute, Basel, Switzerland

Histopathological analysis is a valuable tool to diagnose diseases, to characterize pathomechanisms and to monitor response to treatment. For a range of diseases histopathology is therefore part of the standard diagnostic protocol and treatment may be guided by the results of the analysis. Leprosy is one example, where the classification based on the presence of a set of histopathological features supports the choice of an adjusted treatment regimen. Within the framework of several clinical studies we have performed histopathological and immunohistochemical studies with tissue samples from *Buruli* ulcer patients before, during and after treatment. These analyses have shown that *Mycobacterium ulcerans* is focally distributed in the lesions, with bacteria being mostly present in the deep subcutaneous fat layers and only rarely in the dermal layer of the skin. Immunohistochemical studies for sets of markers demonstrated a remarkable diversity in both the presentation of untreated lesions and in the response to treatment. In combination with clinical features, histopathology may therefore help to develop a more differentiated classification of *Buruli* ulcer lesions. Furthermore immunohistochemical analyses allow monitoring the transition from the inflammatory to the healing phase during treatment of *Buruli* ulcer. Results can give insight into reasons for retarded healing and may support clinical decision making.

1177

STUDYING MYCOLACTONE-TOXIN ASSOCIATED GENE EXPRESSION DURING *BURULI* ULCER INFECTION OF THE HUMAN HOST

Koen Vandelannoote[1], Miriam Eddyani[1], Pieter Bomans[1], Dissou Affolabi[2], Ghislain Sopoh[3], Bouke de Jong[1]

[1]Institute of Tropical Medicine, Antwerpen, Belgium, [2]Laboratoire de Référence des Mycobactéries, Cotonou, Benin, [3]Centre de Dépistage et de Traitement de l'Ulcère de Buruli, Allada, Benin

Mycobacterium ulcerans (MU) is the causative agent of *Buruli* ulcer (BU), a dramatic yet neglected disease found predominantly in tropical riverine regions of West and Central Africa. BU is a chronic destructive infection of subcutaneous tissue where lesions progress from a subcutaneous nodule to an ulcer of increasing size. Despite the extensive tissue damage, little or no inflammatory response occurs at this stage, and lesions are often painless. This unique pathology is attributed largely to the MU-secreted mycolactone, an toxin with cytotoxic, immunosuppressive, analgesic, and potent necrotizing activity, resulting in extensive ulcerative lesions. We hypothesized that higher levels of mycolactone associated gene expression in tissue samples from BU lesions correlate with larger and more advanced BU and predict an increased need for early surgery and worse patient outcome. To investigate this we first optimized RNA stabilisation from mammalian tissues to ensure immediate preservation of both host- and mycobacterial RNA's after surgical excision of affected patient tissues. By dissecting tissues to pieces <1mm immediately after collection and mixing these pieces within 5 minutes with the RNA stabilizer guanidine thiocyanate (GTC), we obtained non-degraded high quality mycobacterial RNA after phenol-chloroform extraction. We then started sampling ulcerative lesions from BU suspected patients, before initiation of antibiotic therapy, and after informed consent was given. Patients were recruited in Allada (Benin) through active and passive case-finding activities in the framework of a parent study to the differential diagnosis of BU. We then determined expression of MU mycolactone associated genes during human infection with quantitative reverse transcription-PCR (qRT-PCR). Relative quantification was performed with different reference genes which allowed accurate internal normalization of qRT-PCR data by geometric averaging of multiple internal control genes. We are currently in the process of assessing the relationship between mycolactone expression and BU lesion stage/patient outcome.

1178

HOSPITAL ACQUIRED INFECTIONS WITHIN THE AMAZON OF PERU

Drake H. Tilley[1], Claudio A. Rocha[1], Rosa Burga[1], Melita Pizango[1], Elia Diaz[2], Cesar Ramal[2], Moises Sihuincha[3]

[1]U.S. Naval Medical Research Unit - 6, Lima, Peru, [2]Hospital Regional Loreto, Iquitos, Peru, [3]Hospital Apoyo, Iquitos, Peru

Increasing bacterial antimicrobial resistance has been observed worldwide and in particular has complicated hospital acquired infections. Although, this problem is well described within developed countries, little is known about nosocomial infections within the Amazon region of South America. In this study, isolates from suspected nosocomial infections were collected from 2 major public hospitals in Iquitos, Peru (Hospital Regional Loreto and Hospital Apoyo) along with pertinent clinical information from June 2011 through April 2013. Over this time period, 381 infections were cultured (229 from Hospital Apoyo and 152 from Hospital Regional) with the most frequent categorized infections being bloodstream infections (37.4%), surgical site infections (25.7%), urinary tract infections (21.7%), pneumonia (19.9%) and skin and soft tissue infections (10.5%). We were able to recover 255 isolates (172 from Hospital Regional and 83 from Hospital Apoyo) to include 63 *Escherichia coli*, 42 *Klebsiella spp.* (17 *K. pneumoniae*), 36 *Pseudomonas aeruginosa*, 31 *Acinetobacter spp.*, 30 *Staphylococcus aureus*, and 53 other. For *P. aeruginosa*, 19% was resistant to imipenem with 45% resistant to ciprofloxacin, however only 8% was resistant to cefepime. Forty-five percent of *S. aureus* was classified as methicillin resistant with only 8% resistance to sulfamethoxazole/trimethoprim. Acinetobacter isolates showed 28% resistance toward imipenem. *Klebsiella spp.* and *E. coli* isolates were found to be multidrug resistant with >80% resistance toward ceftriaxone and sulfamethozazole/trimethoprim and >60% resistance to ciprofloxacin and gentamicin but with almost no resistance to imipenem. In conclusion, multi-drug resistant bacteria from the *Enterobacteriaceae* family predominately complicate hospital acquired infection in Iquitos, Peru with imipenem remaining a reliable treatment for Gram negative infections. However, infection control measures should be employed to prevent further development of antimicrobial resistance within the hospital.

1179

MULTIDRUG-RESISTANT *PSEUDOMONAS AERUGINOSA* OUTBREAK IN A HOSPITAL IN LIMA, PERU

Claudio Rocha[1], Matthew Kasper[1], Enrique Canal[1], Maruja Bernal[1], Juan Carlos Gomez de la Torre[2], Veronica Changano[2], Walter Prudencio[2], Mariana Ramos[1], Drake Tilley[1]

[1]U.S. Naval Medical Research Unit - 6, Lima, Peru, [2]Air Force Hospital, Lima, Peru

As healthcare facilities improve in developing countries, nosocomial infections are of increasing concern. Rapid laboratory based detection allows tracking of the spread of multidrug-resistant pathogens and guides transmissions control measures in the hospital settings. The aim of this investigation was to determine if a series of *Pseudomonas aeruginosa* infections in a military hospital in Lima, Peru were transmitted nosocomially. Swab samples from 4 nosocomial infections were collected by the infection control program at one military hospital in Lima. Samples were processed using conventional bacterial culture and antimicrobial susceptibility testing. The 4 isolated *P. aeruginosa* strains were sub-cultured and subjected to rep-PCR amplification and fingerprinting using the Biomerieux Diversilab bacterial kit. Genotyping results from the DiversiLab determined the level of epidemiological concordance between the strains. The 4 patients ranged in age from 50 to 72 and had spent 15-240 days within the hospital prior to infection. 3 samples were taken from

patients with nosocomial infections related to surgical procedures and the remaining sample was taken from a groin of patient with complicated skin infection. No more samples were taken seeking source of transmission. Of the 4 pseudomona infections, one patient died, two patients were treated with only supportive care with complete remission of the infection, and the remaining patient had colonization. Sub-cultures isolated multidrug-resistant *Pseudomona aeruginosa* from each patient resistant to amikacin, cefepime, ceftazidime, ciprofloxacin, meropenem. All isolates were non susceptible to aztreonam. The Diversilab system determined that all isolates were greater than 96% related, strongly suggesting nosocomial transmission among these patients capable of causing different clinical outcomes, including dead. In this case, fingerprinting using the Diversilab system proved to be a valuable identification tool in the investigation of a nosocomial outbreak of *Pseudomona aeruginosa.*

1180

TRANSMISSION OF BRUCELLOSIS IN SLAUGTHERHOUSE WORKERS AND MILKERS IN THE MUNICIPALITY OF MONTERIA, CORDOBA, COLOMBIA

Gustavo E. Quintero[1], Virginia Rodriguez[1], Alfonso Calderón[2], Cindy Barrios[1], Maria F. Yasnot[1]

[1]Grupo de Investigaciones Microbiológicas y Biomédicas de Córdoba, Universidad de Cordoba, Monteria, Colombia, [2]Instituto de Investigaciones Biológicas del Tropico, de Córdoba, Universidad de Cordoba, Monteria, Colombia

Brucellosis is a bacterial zoonosis, considered occupational disease. In Colombia there are few studies of transmission to humans with temporally and geographically isolated data. The people risk group is composed by slaughterhouse workers, milkers, veterinarians and bacteriologists who process the samples in the laboratory. Traditionally, the diagnosis has been made by Rose of Bengal and confirmed by competitive ELISA or isolation of pathogen. The Rose of Bengal test is not sufficiently specific (54%), the competitive ELISA is very expensive and pathogen isolation is time-consuming and delayed. Establish the prevalence of brucellosis in slaughterhouse workers and milkers in the municipality of Monteria, Cordoba, Colombia. Blood samples from 162 workers were analyzed by Rose of Bengal and competitive ELISA assay. A survey to determine the degree of knowledge and attitudes that have volunteers about the problem was applied. It was found that 9 samples were positive by Rose of Bengal, these sample were confirmed by competitive ELISA. Only one positive sample was found by this method, corresponding to a prevalence of 0.7% of the studied population. The rate of brucellosis in the studied population is 704 per 100,000 inhabitants, despite the knowledge that about the brucellosis have both slaughterhouse workers and milkers.

1181

AEROBIC BACTERIAL CAUSES OF WOUND INFECTIONS IN PATIENTS AT A CAMBODIAN SURGICAL CENTER FROM 2011-2013

Gavin W. Ford[1], Steven Newell[1], Jim Gollogly[2], Michael Prouty[1]

[1]U.S. Naval Medical Research Unit - 2, Phnom Penh, Cambodia, [2]Children's Surgical Center, Phnom Penh, Cambodia

Microbial infections delay wound healing, but the identification of pathogenic bacteria in infected wounds is often unknown. In this study, anonymous wound specimens were obtained from Children's Surgical Center, a Non-Governmental-Organization Hospital located in Phnom Penh, Cambodia. The main objective of this study was to use standard culture and molecular based identification methodologies to discover the prominent populations of pathogenic bacteria associated with wounds in Cambodia and characterize their antibiotic resistance profiles. This study represents an initial effort to develop longitudinal wound culture information across Cambodia to provide estimates of causative agents, evolving antimicrobial-resistance patterns (with particular emphasis on Multi-Drug Resistant Organisms (MDROs)), and the relative proportion of various genotypes for isolated MDROs. Genotypic analysis of selected isolates of interest will be performed in the near future. Specimens from open and closed wounds from a wide variety of anatomical locations, and rarely from sterile body sites, were received from October 2011 to April 2013. Specimens were sent to NAMRU-2 PP based on the clinical judgment of CSC surgeons and not on any set criteria. NAMRU-2 PP was not given any clinical information except for a brief description of the specimen's anatomical source. A total of 252 specimens were collected with 176 positive and 75 negative results by standard bacterial culture, respectively. *Staphlycoccus aureus* was the most frequently isolated bacteria with significant resistance to Methicillin demonstrated. A host of other clinically important Gram positive and Gram negative bacteria were also isolated. Significant resistance to extended spectrum beta-lactam antibiotics was demonstrated among clinically important Gram negative bacteria. The only observed resistance to carbapenems was observed among two isolated strains of *Acinetobacter spp.* Culturing for anaerobic bacteria was not performed. The results of this study demonstrated a significant presence MDROs at one hospital in Cambodia.

1182

ESTABLISHING A PREVALENCE STUDY OF GI PATHOGENS IN A GUATEMALAN COMMUNITY: RESULTS FROM THE PILOT STUDY

Erin J. Stirling[1], **Laura Greisman**[2], Craig A. Sinkinson[3], Ricardo Izurieta[4]

[1]Griffith University School of Medicine, Southport, Australia, [2]Stanford School of Medicine, Stanford, CA, United States, [3]Mayan Medical Aid, Inc., Santa Cruz, Guatemala, [4]University of South Florida College of Public Health, Tampa, FL, United States

Gastrointestinal pathogens account for a large burden of disease in developing countries. *Ascaris lumbricoides* infection can lead to malnutrition, cognitive delay and intestinal perforation. *Helicobacter pylori,* meanwhile, can result in chronic gastritis and gastric carcinoma in long-term infection. The interaction between these common pathogens is unclear at present, however it has been postulated that helminthiasis alters the inflammatory response to *H. pylori* and decreases gastric cancer risk. Estimates of the prevalence of *A. lumbricoides* in Guatemala range from 17.1% to 40%. More data is required on the prevalence of *H. pylori* in Guatemala, however one study in children revealed a rate of 20% and Guatemala reports the second highest rate of gastric cancer in the world. This pilot aimed to gather preliminary data on the prevalence of the two pathogens. Patients over one year and attending the Mayan Medical Aid clinic (Santa Cruz, Atitlan) were selected. A survey was completed before routine clinic visits. Fresh stool samples were brought to the clinic and infection was determined using fecal smear and rapid antigen test (for *A. lumbricoides* and *H. pylori* respectively). Participants returned within 7 days to obtain test results and treatment. 73 patients were enrolled in the study and completed the initial survey, with 56 (77%) returning stool samples. Of these, 57% returned the following week for follow-up. 5/50 (10%) samples tested positive for *A. lumbricoides* whilst 14/52 (27%) tested positive for *H. pylori*. The average age of *A. lumbricoides* cases was 13 years (median 2). Conversely, the average age of *H. pylori* cases was 23 years (median 23). No cases of co-infection were detected. These results may reflect higher *A. lumbricoides* prevalence among children and higher *H. pylori* prevalence among adults. It may be necessary to include *A. lumbricoides* immune tests in future studies to determine the interactions between these two pathogens. Subjectively, the study was well accepted in the community and future large-scale prevalence studies would be viable.

1183

INFLUENZA ILLNESS AMONG CASE-PATIENTS WHO MEET THE SEVERE DENGUE CASE-DEFINITION, EL SALVADOR, 2012

Rafael A. Chacon

Universidad del Valle de Guatemala, Guatemala, Guatemala

Concurrent infection influenza-dengue was described during the 2009-10 influenza pandemic. Was no systematic detection and, in some cases, unspecific diagnostic tools were used. Our objective was to estimate the prevalence of concurrent infection by influenza and dengue virus and identify influenza cases among suspected cases of dengue in patients hospitalized for severe acute respiratory infection (SARI), or suspicion of dengue in the Salvador. We studied 321 subjects of all ages hospitalized who complied with either the severe acute respiratory infection (SARI) case definition or the severe dengue case definition and sought care at influenza sentinel hospitals in El Salvador during July 5-September 30, 2012. This period is considered higher activity of both viruses in the country. All cases were tested for influenza and dengue viruses at El Salvador's National Influenza Center using real time RT-PCR. Ten percent (10%) of all SARI cases and nineteen percent (19%) of all severe dengue cases were positive for influenza virus and negative for dengue virus. One percent of participants had co-infections with influenza and dengue viruses. Co-infection occurred more frequently among dengue case-patients than among SARI case-patients (1.6% vs. 0.5%). It has been shown that concurrent infection of influenza and dengue occurred in the period of greatest viral circulation of both viruses in El Salvador, and outside the framework of the pandemic. We also found that influenza is an important differential diagnosis to consider in patients hospitalized for suspected dengue. Based on these findings we recommended consider empirically treating SARI case-patients with oseltamivir within 48 hours of symptom onset during influenza season, even if they also meet the severe dengue case-definitions. Also consider taking nasal swabs for all severe dengue cases hospitalized during influenza season. It can increase the number of influenza positive cases admitted in hospital.

1184

A RARE CASE: ACUTE TRANSVERSE MYELITIS FROM GNATHOSOMIASIS

Pongdej Wichianprasat, Chayasin Mansanguan, Noppadon Tangpukdee, Srivicha Krudsood

Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

The cutaneous gnathostomiasis is the commonest parasitosis in Southeast Asia but the neurological gnathostomiasis is quite rare. This is a rare case of spinal cord gnathosomiasis. An 86 years old, Thai man presented with sudden and rapid progression of paraplegia and sensory loss below the nipple line with acute urinary retention for 2 hours before consultation. This patient had never eaten raw meat and fish and also had no history of migratory swelling. The physical examination revealed conscious, coherent, oriented, without cardiopulmonary distress; intact cranial nerves; paraplegia of both lower extremities; decreased sensation below T4 level; with no response of deep tendon reflex and absent of Babinski's reflex. The complete blood count revealed no eosinophilia. Blood biochemistry tests, urinalysis, stool examination, chest X-ray and plain whole spine X-ray did not show any abnormalities. However, the Magnetic Resonance Imaging showed hypersignal T2W without enhancing at thoracic level which was diagnosed either neuromyelitis optica or acute myelitis. The lumbar puncture was performed with the following results: WBC 3 cells/uL, RBC 50 cells/uL, and normal protein and sugar levels. Both cerebrospinal fluid (CSF) and serum for *Gnathostoma* antibody (Western Blot) were positive. Albendazole 400 mg/day for 14 days and pulse methylprednisolone 1 g/day for 5 days was started. He gradually improved sensation and bowel and bladder movement, while motor strength had little improvement to grade II/V.

1185

EVALUATION OF WEST NILE VIRUS AND EPSTEIN-BARR VIRUS AS CAUSES OF ACUTE MENINGITIS AND ENCEPHALITIS IN THE COUNTRY OF GEORGIA

Tamar Akhvlediani[1], Emily Rowlinson[2], Margaret Farrell[2], Marcos C. Bautista[3], Tinatin Kuchuloria[1], Nino Akhvlediani[1], Moustafa Abdel Fadeel[2], Tengiz Tsertsvadze[4], Roman Shakarishvili[5], Paata Imnadze[6], James Sejvar[7], Matthew Hepburn[8], Guillermo Pimentel[2], Robert Rivard[9]

[1]Walter Reed Army Institute of Research/United States Army Medical Research Institute for Infectious Diseases Clinical Research Unit, Tbilisi, Georgia, [2]Global Disease Detection and Response Program, U.S. Naval Medical Research Unit - 3, Cairo, Egypt, [3]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [4]Scientific Research Center of Infectious Pathology, AIDS, and Clinical Immunology, Tbilisi, Georgia, [5]P. Sarajishvili Institute of Clinical Neurology and Neurosurgery, Tbilisi, Georgia, [6]National Center for Disease Control and Public Health, Tbilisi, Georgia, [7]National Center for Emerging and Zoonotic Infectious Diseases Centers for Disease Control and Prevention, Atlanta, GA, United States, [8]U.S. Army Medical Command (Majority of contribution occurred while Dr. Hepburn was assigned to United States Army Medical Research Institute for Infectious Diseases), Fort Detrick, MD, United States, [9]U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, United States

There is limited information available regarding infectious etiologies of central nervous system (CNS) infections in the country of Georgia. Data on the relative frequency of these pathogens are important for diagnosis, clinical management, and public health decision making. While West Nile virus (WNV) IgG antibodies have been detected in human sera, there have been no serologically or virologically confirmed cases of WNV in Georgia, and while Epstein-Barr virus is a recognized cause of CNS infections in Georgia, the frequency of EBV-related meningitis or encephalitis is unknown. In October 2010, a hospital-based surveillance study was initiated in Tbilisi, Georgia to determine the infectious etiologies of acute meningitis and encephalitis, and to enhance laboratory capacity for the diagnosis of CNS infections. Cerebrospinal fluid (CSF) and serum (acute and convalescent) were collected. ELISA was used to test for WNV IgM in CSF and serum, and EBV IgM in serum only. Of 199 patients enrolled as of April 2013, 111 were children (age six months - 17 years) and 93 were males. The clinical diagnoses for these patients were bacterial meningitis (82), viral meningitis (95), TB meningitis (9), and encephalitis (13). Of the 199 enrolled patients, samples from 129 have been tested. Of these 129 patients, two were borderline positive (titers of 1.06 and 0.079) for WNV, and 17 patients' serum samples were positive for EBV (titers ranging from 1.2 to 8.1). Of the 17 EBV positive patients 12 were clinically diagnosed as bacterial meningitis, two as viral meningitis, and three as viral encephalitis. Of the 17 EBV positive patients nine had a negative acute test. Continued surveillance is important in order to further define the causes of acute CNS infections in Georgia, however the initial findings of this study provide valuable baseline information regarding the current and emerging etiologies of CNS infection in Georgia.

1186

BURDEN, EPIDEMIOLOGY AND SEASONALITY OF CHOLERA AND ROTAVIRUS AMONG PATIENTS WITH ACUTE DIARRHEA IN FOUR HOSPITALS IN HAITI, 2012-2013

Maria W. Steenland[1], Gerard A. Joseph[2], Lucien Mentor Ali Ber[2], Nicole Freeman[2], Marisa A. Hast[1], Josiane Buteau[2], Benjamin L. Nygren[1], Eyal Leshem[1], Roc Magloire[3], Eric D. Mintz[1], Deborah F. Talkington[1], John Vertefeuille[1], S. Arunmozhi Balajee[1], Jacques Boncy[2], Mark A. Katz[4]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]National Public Health Laboratory, Port-au-Prince, Haiti, [3]Direction of Epidemiology and Laboratory Research, Ministry of Public Health and Sanitation, Port-au-Prince, Haiti, [4]Centers for Disease Control and Prevention, Port-au-Prince, Haiti

An outbreak of cholera began in Haiti in October 2010, and by March 2013 had caused over 650,000 reported cases. All patients with acute watery diarrhea are considered cholera patients for surveillance purposes, but laboratory testing is not performed systematically. Although little is known about the burden of rotavirus in Haiti, national introduction of rotavirus vaccine is planned for 2013. In April 2012, we initiated laboratory-enhanced surveillance for diarrheal disease in patients admitted to 4 Haitian hospitals in 3 departments: West, Artibonite, and Southeast. Nurses collected stool specimens and administered a questionnaire to patients with ≥3 episodes of acute watery diarrhea per day and onset in the past 7 days, who had not received antibiotics. Stool was sent to the National Public Health Laboratory, cultured for *Vibrio species*, *Salmonella* and *Shigella*, and tested for rotavirus by ELISA. From April 3, 2012 – February 27, 2013, we collected specimens from 1,484 patients. The mean age was 27 (range: <1 month – 95 years), 47% were female and 86% were treated in Cholera Treatment Facilities (CTFs). Overall, 973 (65.6%) specimens yielded *V. cholerae*, 8 (0.5%) *Shigella*, and 6 (0.4%) *Salmonella*; 43 (2.9%) were positive for rotavirus. Among 281 children <5 years old, 28.5% and 10.7% were positive for *V. cholerae* and rotavirus, respectively, compared to 74.2% and 1.0% in 1,186 patients ≥ 5 years old. Cholera was more common than rotavirus among 120 children admitted to CTFs (57.5% vs. 6.7%), but not among 150 children admitted to pediatric wards (6.0% vs. 13.3%). Of 973 *V. cholerae* isolates, 970 (99.7%) were serotype Ogawa and 3 (0.3%) were serotype Inaba. The proportion of specimens yielding *V. cholerae* ranged from 43% – 80% per month and was highest during May—June and November—December, 2012. The proportion positive for rotavirus per month ranged from 0 – 17% and was highest in February, 2013. In Haiti, cholera continues to be a major cause of hospitalizations for diarrhea in children and adults, and rotavirus is a common cause of diarrhea among children <5.

1187

VEGFR3 AND OTHER ANGIOGENIC/LYMPHANGIOGENIC GENES ARE ASSOCIATED WITH DEVELOPMENT OF LYMPHEDEMA OF FILARIAL ORIGIN

Linda Batsa Debrah[1], Anna Albers[2], Alexander Y. Debrah[3], Lydia Lust[2], Jubin Osei-Mensah[1], Felix Brockschmidt[4], Tim Becker[5], Christine Herold[5], Ute Klarmann[2], Achim Hoerauf[2], Kenneth Pfarr[2]

[1]Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR), Kumasi, Ghana, [2]Institute for Medical Microbiology, Immunology and Parasitology, University of Bonn, Bonn, Germany, [3]Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana, [4]Department of Genomics, Life and Brain Center, Bonn, Germany, [5]Institute of Medical Biometry, Bioinformatics and Epidemiology, University of Bonn Medical Center, Bonn, Germany

Pathologies of lymphatic filariasis such as lymphedema (LE) and hydrocele are observed in a subset of individuals in endemic areas even though all inhabitants in an endemic community have equal chance of being inoculated with the parasite. While there is ample evidence suggesting that pathology of filarial diseases have genetic propensity, very little work has been done to address it. Recent studies on the molecular mechanisms controlling the lymphatic vessels have shown that vascular endothelial growth factors control lymphangiogenesis in humans by activating the VEGF receptor-3 (VEGFR3) which is principally restricted to the lymphatic endothelium in adults. We showed recently that soluble VEGFR3 in the blood correlated with development of LE and reducing it with doxycycline treatment has ameliorating effect on LE. However, its genetic basis was not known. To assess the role of VEGFR3 and other lymphangiogenic/angiogenic polymorphisms in LE development, a cross-sectional study was designed to genotype 1303 unrelated Ghanaian volunteers comprising 266 lymphedema patients, 691 infected patients but without pathology and 346 endemic controls for single nucleotide polymorphisms (SNPs). In all, 132 SNPs from 64 genes were genotyped and analyzed. A single marker analysis done showed associations with 7 SNPs which included: VEGFR3 [P=0.04], insulin like growth factor-1 (IGF-1) [P=0.03], matrix metallopeptidase 2 (MMP-2) [P=0.03], nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha (NFKB- inhibitior alpha) [P=0.001], carcinoembryonic antigen-related cell adhesion molecule 1(CEACAM-1) [P=0.03], tissue inhibitor of metallopeptidase 2 (TIMP) [P=0.004] genes and interleukin 17 [P=0.05]. Apart from the interleukin 17, all the other mentioned genes were associated with lymphangiogenic/angiogenic modulations, which is a hint that lymphedema development may be associated with lymphangiogenic/angiogenic factors.

1188

THE IMPACT OF THE RAMADAN PERIOD ON CLINICAL PARAMETERS OF PATIENTS SEEN AT A DIARRHEAL HOSPITAL IN URBAN DHAKA, BANGLADESH, 1996-2012

Daniel T. Leung[1], Sumon K. Das[2], M. A. Malek[2], Firdausi Qadri[2], A. S. Faruque[2], Edward T. Ryan[3]

[1]Massachusetts General Hospital/Harvard, Boston, MA, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [3]Massachusetts General Hospital/Harvard, Boston, MA, United States

Ramadan is a month in the Islamic calendar when a significant portion of the global community does not drink or eat from before sunrise to sunset. We hypothesized that such changes may either impact the identity of infecting enteric pathogens, or affect health seeking behavior once ill. To investigate this, we used prospectively-collected data from the Diarrheal Disease Surveillance System of the Dhaka Hospital of the icddr,b in Bangladesh, where 90% of the population are Muslims. For the years 1996-2012, we compared the etiology and clinical presentation of patients who presented with diarrhea during the Ramadan period with patients who presented during control periods, defined as the 30-day period immediately prior to Ramadan. Infecting pathogens were largely the same, although conventional stool cultures obtained from non-Ramadan patients were more likely to yield *Shigella spp.* than during Ramadan periods. Among adult Muslims, Ramadan patients were more likely to complain of severe thirst on arrival compared to controls, though there were no significant differences in rates of severe dehydration between groups. Since cholera is endemic in this area, and can have acute onset that can rapidly lead to severe dehydration, we performed a cholera-based sub-analysis, comparing adult Muslim patients with confirmed *Vibrio cholerae* O1 or O139 infection who presented during Ramadan with those who presented during control periods. Cholera patients who presented during Ramadan were older and more likely to complain of severe thirst. Those who arrived after sunset during Ramadan had a shorter duration of diarrhea, and higher rates of severe thirst, drowsiness, and severe dehydration compared to those arriving after sunset during control periods. These differences were not seen when comparing daytime Ramadan and daytime control arrivals. Our findings suggest that Ramadan may affect both the profile of enteric pathogens, and clinical features of those seeking medical care for diarrhea during Ramadan.

1189

CLUSTER INVESTIGATION OF MELIOIDOSIS CASES REVEALS EVIDENCE OF ENDEMICITY IN PUERTO RICO

Thomas J. Doker[1], Tyler M. Sharp[2], Brenda Rivera-Garcia[2], Janice Perez-Padilla[2], Esther M. Ellis[2], Jazmin Roman Sierra[3], Chanis M. Mercado Olavarria[3], Dana L. Haberling[1], Sean V. Shadomy[1], Mindy Glass Elrod[1], Alex Hoffmaster[1], Jay E. Gee[1], Marta A. Guerra[1], Carmen C. Deseda[3], David D. Blaney[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Centers for Disease Control and Prevention, San Juan, PR, United States, [3]Puerto Rico Department of Health, San Juan, PR, United States

Six locally-acquired melioidosis cases have been identified in Puerto Rico since 1982; of these, four (67%) were fatal. We conducted cluster investigations of the most recent cases (2010 and 2012) to determine likely locations of exposure and identify risk factors for seropositivity among case-patient contacts. Neighbors, co-workers, and social contacts of case-patients were interviewed to collect demographic data, behavioral practices, and past and current medical history. Serum samples were tested for evidence of prior exposure to *Burkholderia pseudomallei* by indirect hemagglutination (IHA); seropositive was defined as IHA titer ≥1:40. For the 2010 case, three (7%) of 43 neighborhood contacts and zero (0%) of eight coworkers were seropositive. For the 2012 case, who was an intravenous drug user (IVDU), 13 (23%) of 56 neighborhood contacts, zero (0%) of one co-worker, and two (67%) of three IVDU contacts were seropositive. Risk factors significantly associated with seropositivity were having had wounds, sores or ulcers (Odds Ratio [OR] =4.7; 95% confidence interval [CI]: 1.3-17.0) and current or past use of illicit drugs (OR=4.2; 95% CI: 1.3-14.0), specifically cocaine (OR=9.1; 95% CI: 1.4-59.0). Traditional risk factors for developing disease such as age, travel history, smoking, alcohol consumption, co-morbidities, and soil and water exposures were not associated with seropositivity. Although only sporadically reported since 1982, the high rate of seropositivity identified in this investigation suggests regional endemicity of *B. pseudomallei* in Puerto Rico. Additional studies are planned to conduct spatial analysis of geospatial data and to identify additional cases and infection distribution through serologic examination and hospital chart reviews. Awareness campaigns addressing melioidosis should be developed for clinicians, laboratories, and high risk populations to promote early recognition and appropriate treatment to help reduce morbidity and mortality.

1190

MANAGING ACUTE FEBRILE ILLNESS IN THE COMMUNITY - IMPLICATIONS FOR POLICY AND PRACTICE IN THE ERA OF RAPID DIAGNOSTICS TESTS FOR MALARIA

Karin Källander[1], Shifra Agaba[1], Edmound Kertho[2], Denis Mubiru[2], James Ssetikoleeko[2], Helen Counihan[1]

[1]Malaria Consortium Africa, Kampala, Uganda, [2]Malaria Consortium Uganda, Kampala, Uganda

Febrile illness like malaria and pneumonia are among the leading causes of child death in Sub-Saharan Africa (SSA). Fever in the tropics has often been considered to be primarily due to malaria and has been treated as such. Recently the treatment guidelines have been further elaborated, recommending parasitological diagnosis before treatment with antimalarials. Pneumonia is still diagnosed presumptively based on symptoms of cough or difficult breathing with fast breathing. At community level, malaria rapid diagnostic tests (mRDTs) were included in Integrated Community Case Management of sick children (ICCM) algorithms where community health workers (CHWs) classify symptoms of malaria, pneumonia, diarrhoea, and treat with antimalarials, antibiotics, oral rehydration solution (ORS) and zinc. The overall aim of this study was to document the diagnoses and treatments prescribed to children under five by CHWs under routine ICCM in Uganda. Patient registries for Jan-Dec 2012 were collected from 106 CHWs in 5 districts in Mid West Uganda. The proportion of child consultations where the child was diagnosed with malaria, pneumonia or diarrhoea was calculated. Quality of care was assessed per each sick-child episode based on treatment provided. A total of 4515 child consultations were documented. Out of these 70% presented with fever and 52% were malaria positive (78% of all fever cases). 48% of children had symptoms of pneumonia, of which 35% were also positive for malaria. Approximately half of the children with pneumonia had fever. 13% were diagnosed with diarrhoea, whereof 29% also were positive for malaria and 34% had pneumonia. Only 2% had overlapping symptoms of diarrhoea, malaria and pneumonia. Of the children with confirmed malaria, 67% were treated with artemisinin combination therapy (ACT); 92% of children with pneumonia received amoxicillin; and 83% of diarrhoeas received ORS and zinc. Only 10% of children with a negative malaria test were prescribed ACT and 6% of children without fast breathing were prescribed antibiotics. With the rapid expansion of ICCM across countries in Africa, the consideration whether to include mRDTs in the clinical algorithm is key. Our findings show that symptom overlap is common and the majority of children receive more than one diagnosis. mRDTs reduced unnecessary malaria treatment and likely increased appropriate treatment of children with non-malaria fever. Correct treatment by CHWs was high.

1191

TOSCANA VIRUS INFECTIONS IN MARSEILLE, FRANCE: A STUDY OF SEVENTEEN CLINICAL CASES WITH LABORATORY DOCUMENTATION

Julien Dupouey[1], Laurence Bichaud[1], Laetitia Ninove[1], Christine Zandotti[1], Laurence Thirion[1], Xavier de Lamballerie[1], **Remi N. Charrel**[2]

[1]Aix Marseille Univ - IRD French Institute of Research for Development - EHESP French School of Public Health, Marseille, France, [2]Aix Marseille University, Marseille, France

Toscana virus (TOSV) is a sandfly-borne phlebovirus (Phlebovirus, Bunyaviridae), discovered in 1971 in Central Italy, principally transmitted by *Phlebotomus* perniciosus and Ph. perfiliewi. TOSV is considered as an emerging virus in Portugal, Spain, France, Italy, Greece, Cyprus, Croatia, and Turkey. Most of the clinical reports of TOSV infections are case reports, and few studies considered series of cases. Moreover, the laboratory documentation of suspect cases is rarely analysed by using the WHO criteria for arboviral infections (2012 Case Definitions: Nationally Notifiable Conditions Infectious and Non-Infectious Case. (2012). Atlanta, GA: Centers for Disease Control and Prevention). TOSV infection was diagnosed based on virus isolation (n=1), RT-PCR (n=6), TOSV IgM (n=15) and or seroconversion (n=6). Here, we report a series of 17 clinical cases (15 confirmed, 2 probable) of TOSV infection recorded in the Virology laboratory of the Public Hospitals of Marseille between 2004 and 2011. Eleven patients were men (64.7%), and median age was 45 years (range, 4-76). Fourteen TOSV infections (82%) occurred between June and September, and 3 cases in March, April and November; only two patients reported recent travel in Croatia and in Tuscany, respectively. The outcome was favorable in all cases. All cases were symptomatic, and CNS signs were observed frequently: meningitis, meningoencephalitis or encephalitis (n = 10), atypical neuro-muscular symptoms (n = 3) such as hemiparesis, fasciitis, myositis. In conclusion, TOSV infection is a prominent cause of summer febrile illness with or without central or peripheral neurological manifestations in south-eastern France. In our study TOSV was the third cause of CNS infection, after enteroviruses and herpesviruses (HSV and VZV). These findings, together with seroprevalence data, confirm that TOSV is the most frequent arbovirus in France.

1192

CASE REPORT: INFECTION WITH *LEISHMANIA (VIANNIA) LAINSONI* IN LORETO, PERU

Maxy De los Santos[1], Salomon Durand[2], Erika S. Pérez[3], Juan F. Sanchez[1], Moises Sihuincha[4], Carmen M. Lucas[1], G. Christian Baldeviano[1], Kimberly A. Edgel[1], Andres G. Lescano[1]

[1]Department of Parasitology, U.S. Naval Medical Research Unit No. 6, Lima, Peru, [2]U.S. Naval Medical Research Unit No. 6, Iquitos, Peru, [3]Facultad de Ciencias Biológicas, Universidad Nacional Mayor de San Marcos, Lima, Peru, [4]Hospital de Apoyo de Iquitos Cesar Garayar García, Iquitos, Peru

Leishmania (Viannia) lainsoni has been isolated in human samples, sandflies and putative reservoirs in the Amazon Basin of Brazil, in the Yungas of Bolivia, and in the central and southern Peruvian Amazon Basin. However, this species has neither been reported in the northern Peruvian Amazon basin nor other countries further north such as Ecuador. We describe here a recent case of *L. (V.) lainsoni* from a 47 year old male patient in Loreto, Perú. The patient had a 13 x 8 mm ulcer on the forehead close to the right ear presenting with neck lymphadenitis. There was neither mucosal compromise nor previous report of leishmaniasis. The patient has lived in Loreto since 2009, but travels continually for work. Before diagnosis, he worked in Quillabamba, Cuzco (Jul 2012) but only within city limits where there have been no documented reports of leishmaniasis. Then he worked in Coca, Ecuador (Aug-Oct 2012) and finally returned to work in rural areas of Loreto (Oct 2012-Feb 2013) when the lesion appeared. Both microscopy and culture of lesion aspirates were positive for *Leishmania*. PCR for kinetoplastid DNA from a punch biopsy, scraping and filter paper impression confirmed the diagnosis. In addition, our validated nested RT-PCR identified the species as *L. (V.) lainsoni*. The patient received complete treatment with 20 daily doses of intravenous sodium stibogluconate (20mg/kg) over 26 days and achieved 70% lesion healing by the end of treatment. This patient was most likely infected in Loreto, Peru, or Coca, Ecuador, although it cannot be conclusively demonstrated that he was not infected in the Southern Amazon Basin of Peru. Our finding suggests that the known geographical distribution of this uncommon *Leishmania* species is broader than currently suspected, and highlights the need to continue identifying the species of *Leishmania* circulating in the region.

1193

EARLY PARASITOLOGICAL RESPONSE IN PATIENTS TREATED WITH ARTEMISININ COMBINATION THERAPIES IN ASIA: A POOLED ANALYSIS OF INDIVIDUAL PATIENT DATA

Prabin Dahal, on behalf of WWARN ACT Asia Baseline Study Group

WorldWide Antimalarial Resistance Network, Oxford, United Kingdom

Delayed early parasite clearance is the hallmark of artemisinin resistance. The proportion of patients remaining parasitaemic on day 3 is a useful indicator of early parasitological response. The WHO currently uses positivity rates on day 3 exceeding 10% as an alert for a potential decline in artemisinin sensitivity. We investigated the clinical determinants associated with persistent parasitaemia on day 3 and explored the trends of early parasitological response following the initiation of artemisinin-based combination therapies (ACT) treatment in Asian patients. A total of 10,828 individual patient data from 26 efficacy trials (1991-2010) shared with the Worldwide Antimalarial Resistance Network (WWARN) were pooled using standardised methodology. Overall 5362 (49.5%) patients were treated with Artesunate Mefloquine (AS+MQ), 2,726 (25.2%) with Dihydroartemisinin-Piperaquine (DP), 2,415 (22.3%) with Artemether-Lumefantrine (AL), and 325 (3%) with Artesunate-Amodiaquine (AS-AQ). Two major risk factors were identified for being parasitaemic on Day 1, 2 and 3: the log of the baseline parasitaemia and being febrile at enrolment (both $p<0.001$). In multivariable analysis in which study site was fitted as a random effect, the risk of being parasitaemic on day 1 [AOR: 0.93, 95% CI: 0.85-1.01, P=0.10], day 2 [AOR: 0.86, 95% CI: 0.78-0.96, P=0.01] and day 3 [AOR: 0.94, 95% CI: 0.84-1.04, P=0.23] were found to have decreased over time after adjusting for age, baseline parasitaemia (log), initial fever and the treatment received. The risk of being parasitaemic on day 3 did not differ between treatment regimens. However this pooled analysis didn't include data from Thai-Cambodia border where artemisinin resistance has emerged.

1194

INFECTIOUS ETIOLOGIES OF MALARIA-NEGATIVE ACUTE FEBRILE ILLNESS AMONG ADULTS AND CHILDREN IN NORTHEAST TANZANIA

Helena Hildenwall[1], George Mtove[2], Ben Amos[3], Hugh Reyburn[4]

[1]Division of Global Health, Karolinska Institutet, Stockholm, Sweden, [2]National Institute for Medical Research, Amani Centre, Tanga, United Republic of Tanzania, [3]Joint Malaria Programme, Muheza, United Republic of Tanzania, [4]London School of Hygiene & Tropical Medicine, London, United Kingdom

The most recent WHO Guidelines recommend the use of parasitological testing to guide antimalarial drug use in all ages. To support this and guide management of test-negative patients there is an urgent need to know more about aetiologies to non-malarial febrile illness. We aimed to identify the cause of non-malarial fevers in children and adults in a malaria-endemic area of northeast Tanzania. 1087 patients, 3 months to 50 years old, with a history of fever during the past 48 hours were enrolled. A combined throat and per nasal swab, urine sample and aerobic blood culture were taken on each case. Chest X-ray was done in cases with suspected pneumonia. Patients were followed up 2, 7 and 14 days after enrolment to follow progress and to take a convalescent blood sample. Blood and NP-swabs were analysed by culture growth and multiplex PCR to identify illness-causing pathogen(s). Upper respiratory tract infection was the most common diagnosis in children under five years (67%) while adults were most commonly diagnosed with unspecified fever (34%) and urinary tract infection (33%). 8% of children below five were diagnosed with non-severe pneumonia and 4% with severe pneumonia. 46 patients were admitted (4%) and all but five of them were less than five years. There were no case fatalities during the study and 1010 patients were seen on day14 whereof 2% reported no improvement or worsening. 19% of throat/NP cultures were positive with most (63%) growing *S. pneumonia*. Positive growth was more common among children under five (33% positive cultures) compared to older patients (OR 1.63). For urine cultures, 9% of children less than one presented significant bacterial growth, all with *E.coli*. In adults, 6% had significant bacteria in urine, most of them *E.coli* or *klebsiella*. Seven blood cultures flagged positive for *Salmonella typhi*, four children grew *streptococci spp* and two adult blood cultures were positive for *E.coli*. Results from multiplex PCR and X-rays will provide further understanding of the illness causing pathogens and inform management guidelines.

1195

A TROPICAL DISEASE IN CLEVELAND, OHIO: A CASE OF CEREBRAL MALARIA

Jessica A. Kumar[1], Zahra Toossi[2], Sharanie Sims[3], D'Arbra Blankenship[1], Chris King[1]

[1]University Hospitals, Case Western Reserve University, Cleveland, OH, United States, [2]Case Western Reserve University, Division of Infectious Diseases and HIV Medicine, Cleveland, OH, United States, [3]Louis Stokes Veterans Affairs Medical Center, Cleveland, OH, United States

Severe *Plasmodium falciparum* malaria has a high mortality rate often presenting in the first 48 hours after hospital admission, thus correct diagnosis and delivery of appropriate treatment can be life- saving. Artesunate is the recommended treatment but parenteral therapy is only available through the Centers for Disease Control. Providers who do not see this disease severity can often misdiagnose or delay diagnosis.

We present a case of a 67 y/o Caucasian male admitted to the Veteran's Hospital in Cleveland, OH with a past medical history notable for atrial fibrillation presenting with flu-like symptoms, notably diarrhea for the past 3 days after returning from a trip to Uganda where he traveled 6 times previously for mission work. He complained of myalgias, non-bloody emesis, dizziness and chills. He drank only bottled water, had no sick or animal contacts, used mosquito nets and became ill on the plane. The patient did not take malaria prophylaxis nor did he receive any vaccinations prior to travel. On exam he was febrile, tachycardic and tachypneic with no significant physical exam findings. Lab work showed a bilirubin: 2.7, LDH: 562, hemoglobin: 15.9->11.3 and hematocrit: 46.2->32.5, platelets: 87->42, SGOT: 65->93, SGPT: 36 with creatine: 1.2. His blood cultures, HIV, hepatitis and stool cultures were negative. Malaria smear was ordered and atovaquone/proguanil was started. With one dose left before finishing treatment, he had worsening confusion with altered mentation. Labs showed thrombocytopenia and transaminitis. On initial smear he had a 5% *P. falciparum parasitemia* which dropped to 2%. Parenteral Artesunate was requested from the CDC malaria branch. Within in 24hours of starting parenteral Artesunate the patient clinically improved. Whole blood collected and stored in the refrigerator for over 3 days showed a high grade *parasitemia* greater than the patient's smears and the parasite itself, was able to be grown in culture. He had a sustained anemia and there was concern for a post-treatment hemolytic process. We advocate for increased accessibility to parenteral Artesunate therapy, rapid diagnostics and improved malaria educational tools/scoring for clinical assessment and progression to advanced disease for clinicians/house officers in the United States unfamiliar with this disease and severe manifestions such as cerebral malaria as well as the presenting symptoms and clinical course.

1196

INNOVATIVE AND FAST ASSAYS FOR THE DIAGNOSIS OF *SCHISTOSOMA MANSONI* FOR CLINICAL ACUTE AND/OR CHRONIC FORMS

Rafaella F. Queiroz[1], Donald A. Harn[2], Paulo Marcos Z. Coelho[1]

[1]Oswaldo Cruz Foundation, Belo Horizonte, Brazil, [2]University of Georgia, Athens, GA, United States

Control constraints of schistosomiasis include the lack of diagnostic methods with high sensitivity. We initiated a prospective study in southeast Brazil in order to develop sensitive diagnostic methods for *Schistosoma* mansoni infection, with 4 endemic areas together with 80 travelers infected in a freshwater pool. Sera, whole blood, urine and saliva samples from the patients were used for the standardization of innovative diagnostic methods. Comparisons were performed with eggs in feces, IgG titers, encephalomyelitis by NMR and clinical symptoms. The new methods used were immunochromatography (dipstick), Immunomagnetic Separation and ELISA with highly purified monoclonal antibodies. We could diagnose acute patients 10 days post-infection, also more than 95% of positive cases from chronic and low endemicity patients. New methods for IgG detection using purified glycoprotein or recombinant protein or peptides (10 aminoacids) were superior to conventional ELISA. Best results were seen for recombinant protein with 100% of sensitivity. Data showed 100% of sensitivity of chronic patients and 98% of acute patients.

1197

CLINICAL FEATURES OF NON-COMPLICATED MALARIA IN SEMI-IMMUNE INDIVIDUALS OF WESTERN BRAZILIAN AMAZON

Antonio C. Martins[1], Alanderson Ramalho[1], Felipe Araújo[1], Rayanne Arruda[1], Andreus Schlosser[1], Rudi Nogueira[1], Maria Guimarães[1], Cássio Braga[1], Athaid Cayotopa[1], Saulo Mantovani[1], Thasciany Pereira[1], Breno Matos[1], Aline Silva[1], José Filgueira Júnior[1], Wagner Klein[1], Carlos Cavasini[2], Rosely Malafronte[3], Claudia Codeço[4], Mônica da Silva-Nunes[1]

[1]Federal University of Acre, Rio Branco, Brazil, [2]São José do Rio Preto Medical School, São José do Rio Preto, Brazil, [3]University of São Paulo, São Paulo, Brazil, [4]Oswaldo Cruz Foundation, Rio de Janeiro, Brazil

We analyzed clinical characteristics of 115 microscopy and PCR-confirmed, uncomplicated, symptomatic malaria cases diagnosed in urban Amazonians through passive detection in Acre, Brazil. Patients were between 6 years and 92 years (58.2% males), and were highly exposed to malaria (68.1% had between 1 and 10 previous episodes, 29.2% had more than 10 previous episodes and only 2.7% never had an episode before). The most common species was *P. vivax* (73.04%), followed by *falciparum* malaria (25.2%) and mixed-species infection (1.76%). Parasitemia was below 500 parasites/mm^3 in 64% of cases, and only 1% of cases had *parasitemia* higher than 10.000 parasites/mm^3. Eighteen symptoms (fever, chills, sweating, headache, retro-ocular pain, myalgia, arthralgia, abdominal pain, lumbar pain, nausea, vomiting, diarrhoea, dizziness, flu-like symptoms, cough, dyspnea, odynophagia, bitter mouth) were investigated using a structured questionnaire. Headache (89.4%), fever (84.1%) and chills (77.0%) were the most frequent symptoms. Fever was perceived as "intense" in 47.0% episodes, with no fever reported in 15.9% episodes, although other symptoms were present. Falciparum malaria cases were more likely to present with gastrointestinal symptoms, although no statistical significance was obtained. Arthralgia and myalgia were more frequent in older people (100% of upper age quartile against 70% of lower age quartile, $p < 0.01$, Qui-square test) than younger people, while respiratory symptoms were more frequent in the younger (66.7% in the lower age quartile, $p = 0.025$, Qui-square test). Diarrhoea was also more frequent in the upper age (10.7%) than lower age (3.3%) quartile ($p = 0.01$, Qui-square test). The only symptom that correlated with higher peripheral *parasitemia* was dizziness (70.7% versus 48.6%, $p = 0.03$, Qui-square test). No significant association was found between symptoms and sex or previous malaria exposure. These factors are all likely to affect the effectiveness of malaria control strategies based on active or passive detection of febrile cases in semi-immune populations.

1198

BIOMARKERS AND RISK FACTORS FOR INADEQUATE ERYTHROPOIESIS IN ALL-CAUSE PEDIATRIC ANEMIA IN MOZAMBIQUE

Ariel H. Achtman[1], Ruth Aguilar[2], Connie Li Wai Suen[1], Charity W. Law[1], Cinta Moraleda[3], Hendrik Falk[1], Montse Renom[3], Sandra Pilat[1], Augusto Nhabomba[3], Eusebio Macete[3], Ivo Mueller[1], Gordon K. Smyth[1], Pedro L. Alonso[2], Clara Menéndez[2], Louis Schofield[1]

[1]The Walter and Eliza Hall Institute, Melbourne, Australia, [2]Barcelona Centre for International Heath Research, Barcelona, Spain, [3]Manhiça Health Research Center, Maputo, Mozambique

Anemia affects 1.6 million people globally and is massively understudied, particularly as a multifactorial disease. Dysregulation of erythropoiesis is thought to be a major factor in chronic and recurring anemias, but is rarely studied in human populations, because of poor accessibility of bone marrow and limitations of methods for measuring erythropoiesis. We collected 286 bone marrow (BM) aspirates in the context of a large case-control study of anemia in Mozambique and examined a wide range of clinical, hematological, biochemical, immunological, microbiological

and genetic markers. We developed novel flow-cytometric analysis and a transcriptional profile based on erythroblast-specific gene expression to quantify erythropoiesis. Both measures correlated well with classic morphological quantification in stained BM smears (both $p<0.0001$). Whereas peripheral reticulocyte numbers showed no correlation with BM erythropoiesis in this cohort, the red cell distribution width (RDW), a measure of variance in erythrocyte volume, was validated as a novel peripheral biomarker for this important response ($p<0.0001$). Samples were categorized as having low or high erythropoiesis relative to hemoglobin levels. Uni- and multivariate statistical testing revealed that high C-reactive protein levels and *bacteremia* were most highly associated with low erythropoiesis. Hemolysis, shifts in the BM balance of myeloid cells and lymphocytes and high EPO levels relative to the degree of anemia were associated with high erythropoiesis and unexpectedly, so was infection with *Plasmodium falciparum*. The risk factor panel for low erythropoiesis differed from the panel of risk factors for anemia identified in the same cohort, in particular in regards to nutritional deficiencies. Thus, these data provide superior direct and peripheral biomarkers of erythropoiesis, differentiate several groups of erythopoietically suppressed BM and provide a rich data set for our ongoing analysis of transcriptional footprints indicative of underlying mechanisms. Such findings may inform future therapies for this serious disease.

1199

HERPES ZOSTER ASSOCIATED WITH PENTAVALENT ANTIMONY THERAPY FOR TEGUMENTARY LEISHMANIASIS - A 22 CASE SERIES OF A TROPICAL MEDICINE HEALTH SERVICE IN BRAZIL

Marcia Hueb, Andrea B. Barbosa, Alex M. Rodrigues, Cor Jesus F. Fontes

Universidade Federal de Mato grosso - Brasil, Cuiaba, Brazil

The pentavalent antimonial is considered as the first line drug for leishmaniasis treatment in Brazil. Although it has been prescribed for many years, the mechanism of action is not completely understood. It is known antimonial inhibit intracellular glycolysis and fatty acid oxidation, beside link with sulfhydryl groups, resulting in the parasite death. Side effects are frequent and include myalgia, arthralgia, anorexia, headache, vomiting, and even cardiotoxicity, nephrotoxicity and sudden death are possible. Herpes Zoster (HZ) has been rarely described during or shortly after receiving therapy with antimonial. HZ is a secondary clinical manifestation of varicella zoster virus (VZV), an α-herpes virus that seems associated with depressed cellular immunity, and eventually related with antimonial treatment. This paper reports a series of cases of HZ observed during or shortly after antimonial treatment for the various clinical forms of leishmaniasis. Patients reviewed were treated at the infectious disease clinic of a Brazillian University Hospital, between 1995 and 2010. The onset day of HZ was recorded. Out of 21 in a total of 22 patients were male with a median age of 53. Sixteen had Tegumentary Leishmaniasis (LC, LM, Disseminated). N-methyl-glucamine was used in 18 cases and Sodium Stiboglucanate in 4. The median dose of N-methylglucamine was 18.2 mg / kg. The median time to onset HZ was 20 days (25 days for N-methyl and 16.5 to stibogluconate). Although the mechanism by which varicella zoster virus becomes reactivated is not known, the association of zoster with conditions that depress cellular immunity led to suppose that patients under antimonial treatment could be transiently suppressed regarding cellular immunity even considering that older age and high doses of antimony can act as predisposing factors. Most of our patients had tegumentary leishmaniasis and their base clinical conditions cannot be related an immunological suppression.

1200

THE CHANGING FACE OF NOMA IN LAOS

Margaret L. Srour[1], Denise Baratti-Mayer[2], Klaas Willem Marck[3]

[1]Health Frontiers, Muang Sing, Lao People's Democratic Republic, [2]Geneva Study Group on Noma, Service of Plastic and Reconstructive Surgery, Geneva University Hospital, Geneva, Switzerland, [3]Dutch Noma Foundation, Goutum, Netherlands

Noma is an orofacial gangrene and an opportunistic infection, primarily occurring in children whose health is weakened by malnutrition and living in extreme poverty. Acute noma rapidly destroys the face and, without treatment, has a high mortality of 90%. In the acute phase the patient is treated with antibiotics and nutritional support. Survivors of noma are often severely disfigured and functionally impaired (trismus, speech problems, oral incontinence). Surgical rehabilitation of noma patients requires experienced surgical teams. Noma cases have been recorded historically throughout the world, but economic and public health improvements have resulted in the apparent disappearance of noma in developed countries. Most cases of noma occur on in Africa. There are few reports of noma in Asia. Prior to 2002, there were no reported cases of noma in Laos. Since 2002 one patient with acute noma and 36 survivors have been found throughout the country. Visiting surgical teams have successfully treated the majority of these patients. Most are young adults whose disease occurred several decades ago, which suggests that noma is disappearing in Laos. Despite economic growth, chronic malnutrition continues to weaken the health of almost 50% of Lao children with much higher rates in rural areas. These rates have changed little despite economic improvements. The risk factors for noma are malnutrition, poverty, poor hygiene and lack of clean water, lack of breastfeeding, infections especially measles and malaria, close contact with animals, lack of immunizations, vitamin deficiencies and poor health care access. These risk factors continue to threaten the health and well-being of Lao children. Follow-up of Lao noma patients, some for 10 years, reveal that many have required multiple surgeries. This has resulted in improved cosmetic, functional, and psychosocial outcomes. Ultimately, the elimination of noma risk factors should lead to the complete disappearance of this neglected childhood disease.

1201

HISTORICAL OVERVIEW OF STUDIES ON JAPANESE ENCEPHALITIS VIRUS IN NORTHERN VIETNAM, 1964-1978

Do Quang Ha

Oxford University Clinical Research Unit Vietnam, Ho Chi Minh City, Vietnam

In northern Vietnam from April to September every year, children often have had a malignant syndrome called Acute Encephalitis Syndrome (AES). From 1965 to 1977, the total number of AES in children was 22,545 cases, from whom 4,774 died. Before 1964, the etiology of AES in northern Vietnam has not yet been identified; so the JE infection in our country is still a blank area on the World Map. In this study, we have to determine the etiology of the AES by carrying-out: Virus isolation: From 1964 to 1976, we have isolated 22 JEV from different specimens including: 10 from AES children (1 from blood, 9 from brains), 1 from bird, 1 from swine blood, 10 from mosquitoes. The positive diagnoses (HI test) using paired sera taken from AES children have been varied from 25.58% to 67.5%. Investigate the JEV circulation through serological survey of healthy humans: During 1964-1978, we have tested 7,868 sera, from which the positive rate varied from 25% to 82.94%. Vectors transmitting JEV: JEVs were isolated for the first time in 1971 from *Culex tritaeniorhynchus*. Then in 1975-1976, JEV were discovered from *Cx. gelidus, Aedes albopictus* and *Ae. (Sp) diemmaccus*. To this time, *Cx. tritaeniorhynchus* has been identified as the main vector transmitting JEV in northern Vietnam. Explore the circulation of JEV in animals: - Birds: in 1964 we have isolated the strain LD-68 from bird *Garrulax perspicillatus* - Gmelin and have found 8/14 bird species carrying JE antibody; therefore wild birds have played

the role as reservoir of JEV. - Animals: a/ Serological survey in swine: In 1970: 606 pig sera have been collected in several localities, from which 63.69% were positive to JEV with MAT: 1/619.76. b/ Sero-survey in livestock: During 1972-1975, we have continued to investigate sera from other animal species. The results obtained demonstrated that swine was the most commonly infected with JEV to compare with other domestic animals. With the results obtained, we asserted that northern Vietnam was a natural focus of JEV. This virus virtually persisted everywhere, in wild birds, an arthropod transmitted this virus from bird to livestock; at first to a few pigs, *Cx. tritaeniorhynchus* sucking blood from pigs in phase of viremia then widespreading this virus to other pigs, allowed herd of infected pigs increased rapidly and just to this phase, JE infections occur in humans with a small number of children suffered from AES .

1202

STRUCTURAL COMPARISON OF THE ANTIGENIC CHARACTERISTICS OF USUTU VIRUS AND WEST NILE VIRUS ENVELOPE PROTEINS

Birgit Nikolay[1], Amadou A. Sall[1], Cheikh S. Boye[2], Tim Skern[3]

[1]Institut Pasteur de Dakar, Dakar, Senegal, [2]Universite Cheikh Anta Diop, Dakar, Senegal, [3]MFPL- Medical University Vienna, Vienna, Austria

Usutu virus (USUV), a flavivirus belonging to the Japanese encephalitis serocomplex, was isolated for the first time from mosquitoes in South Africa in 1959. The virus emerged in Europe in 2001 and caused severe morbidity among two patients in Italy in 2009. A recent study describing the genetic diversity among USUV isolates from Africa showed the existence of an outlier subtype strain named CAR_1969. Cross-reactions observed in serological assays between USUV, CAR_1969 and West Nile virus (WNV) indicate that these viruses share antigenic characteristics amongst their envelope (E) proteins. Therefore, the comparison of their E proteins might provide important information about USUV pathogenesis and immune reactions to the virus in the human body. We investigated the molecular background of the observed cross-reactions by comparing the E protein sequences of seven USUV strains, USUV subtype strain CAR_1969 and WNV strain 2471 with focus on the binding site defined by the WNV neutralizing antibody E16. USUV SouthAfrica_1959 differs from WNV 2741 in three of four residues critical for E16 antibody binding and five of 12 additionally involved residues. In contrast, USUV subtype CAR_1969 differs from WNV 2741 in two critical residues and five additional residues. Furthermore, USUV subtype CAR_1969 differs from other USUV strains in two critical residues. As E16 antibody binding has previously been shown to be highly specific for WNV, the variation in amino acid residues suggests that the region corresponding to the WNV E16 epitope is probably not involved in the observed cross-reactions and might however partially explain the different antigenic characteristics. Nevertheless, as a therapeutic effect of E16 antibody has been described in WNV infected mice, a USUV specific antibody generated against the region corresponding to the WNV E16 binding site might therefore represent an interesting approach for the treatment of USUV infections.

1203

JAPANESE ENCEPHALITIS OUTBREAK IN WESTERN BANGLADESH, 2011

Hossain M. Sazzad[1], M. Jahangir Hossain[1], Stephen P. Luby[2], Mahmudur Rahman[3], Susan Hills[4], Marc Fischer[4], James Sejvar[5], Barbara W. Johnson[4], Emily S. Gurley[1]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [2]Global Disease Detection Branch, Division of Global Health Protection, Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Institute of Epidemiology, Disease Control and Research, Dhaka, Bangladesh, [4]Arbovirus Branch, Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, Fort Collins, CO, United States, [5]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

Acute meningo-encephalitis (ME) surveillance was initiated in October 2007 in four Bangladeshi hospitals, including Rajshahi Medical College Hospital (RMCH) in western Bangladesh. RMCH identified 550 ME cases from October 2007 to December 2008; 33 (6%) were caused by Japanese encephalitis (JE) virus infection. Through the Bangladesh government's event-based surveillance, all Bangladeshi hospitals are required to report unusual increases in ME admissions. In mid-October to mid-November 2011, Kushtia General Hospital (KGH) in western Bangladesh reported an increase in ME admissions over the 0-2 ME cases they usually admit each month. The objective of this investigation was to confirm an ME outbreak and identify the etiology. Both surveillance systems enlisted febrile cases with evidence of acute neurologic dysfunction, including altered mental status. RMCH identified cases throughout 2011, while KGH identified cases from mid-October to mid-November 2011. Physicians collected blood and cerebrospinal fluid (CSF) samples which were tested for JE virus (JEV)-specific IgM. We defined confirmed JE cases as those with detectable IgM against JEV in serum and/or CSF by ELISA. In 2011, RMCH identified 320 ME cases and collected serum and/or CSF samples from 281 (88%). Of these, 39 (14%) had evidence of JEV infection. Median age of JE cases at RMCH was 40 years (range: 4 months - 72 years); 28 (72%) were above 16 years old; 29 (74%) were male. Six (15%) died. 24(62%) JE cases had onset of illness during October to November 2011. The proportion of serum and/or CSF tested ME cases with JE at RMCH was significantly higher in 2011 than 2008 [3% (12/434)], 2009 [2% (11/489)], and 2010 [4% (10/267)] ($p<0.001$ for each year). From mid-October to mid-November 2011, 14 ME cases were identified at KGH. Four cases had detectable IgM against JEV and seven cases did not; although three of them were negative 7-9 days after onset of illness; three case-patients died before blood samples were collected. Median age of confirmed JE cases admitted to KGH was 50 (range: 40 - 60) years; two were male. In conclusion, the high proportion of JE cases among tested ME cases in 2011 in western Bangladesh suggests a JE outbreak. The high proportion of JE cases among adults with ME suggests JE is an emerging infection in this region. The national vaccination programme should evaluate the feasibility of JE vaccination among residents of western Bangladesh.

1204

QUASISPECIES ANALYSIS OF HEPATITIS C VIRUS IN MOTHER-TO-CHILD *IN UTERO* TRANSMISSION

Tamiris T. Dias[1], Sione S. Passini[1], Maria A. Zarife[2], Mitermayer G. Reis[1], Luciano K. Silva[1]

[1]Gonçalo Moniz Research Center - FIOCRUZ-BA, Salvador, Brazil, [2]Central Public Health State Laboratory (Lacen-BA), Salvador, Brazil

Mother-to-child-transmission (MTCT) is the most common cause of hepatitis C virus (HCV) infection in children. This study aimed to evaluate viral factors implicated in HCV MTCT. Four HCV-infected pregnant women and one HCV-infected mother-newborn pair were included in this study. Sequences were obtained from the regions 5'UTR, E1, HVR1, E2 and NS5B by direct PCR product sequencing and cloning. Quasispecies diversity was

analyzed by different parameters (clonotype ratio, mutation frequency, Pn and normalized Shannon entropy), comparing (1) MTCT+ vs MTCT- groups, and (2) mother-newborn pair. A framework was used to establish association between nucleotide frequency and MTCT. Two cases of MTCT were identified, but a sample from only one newborn was available. Viral loads from all subjects were above the quantification limit. Both cases of MTCT belonged to genotype 1a and only this subtype was further analyzed. Direct sequencing from PCR products did not reliably represent the quasispecies complexity and was not used. There were no coincident clonotypes between MTCT+ and MTCT- groups, except for 5'UTR. At the amino acid level, mother and newborn shared only the master clonotype. All minor clonotypes were exclusive. Higher quasispecies diversity was observed within E2 and NS5B regions. HVR1 presented the lowest diversity within the coding region. Quasispecies diversity from the MTCT+ group was always greater than seen in the MTCT- group; however, no statistically significance was observed. Thirty-five mutations in the coding regions were significantly associated with MTCT. Data from the mother-newborn pair suggest that the *in utero* transmission occurred in an earlier time point of the pregnancy and that the virus probably crossed the placental tissue leading to a bottleneck. Quasispecies diversity was not associated with MTCT but the presence of significant mutations along the coding region suggests that the whole genome contributes to the ability of *in utero* transmission. Further studies are required to establish if these variants could be useful to predict MTCT.

1205

CLUSTER OF MEASLES CASES AMONG FARMERS CHILDREN, GWABABAWA LOCAL GOVERNMENT AREA, SOKOTO STATE, NIGERIA - 2012

Measles, a highly infectious vaccine preventable disease, associated with high morbidity and mortality. Measles is the fifth leading cause of death among children younger than 5 years in Nigeria. Northern Nigeria suffers recurrent outbreaks due to low immunization intake. On 9 June 2012 we investigated a suspected measles outbreak in Gwadabawa district to identify risk factors and institute control measures. An unmatched Case-Control study was conducted. A case was a person less than five years residing in Gwadabawa presenting with fever, generalized maculopapular rash, plus any of the followings, Fever, Cough, Conjunctivitis, or Coryza between 1 June and 1 July 2012. Neighbourhood controls were used. We administered questionnaires to obtain information on Risk factors, socio-demographic characteristics, and immunization status. Fifteen Blood samples were collected. We interviewed 63 cases and controls 63 controls. The mean age was 25 Months (SD of 14.4) nine samples tested positive to measles IgM. Being vaccinated with one dose of measles and having a national Vaccination card was found to be protective (OR=0.0182, 95% CI: [0.009-0.3532] and OR=0.0067 CI: 0.0059-0.7550] Disease was associated with a history of contact with a known measles case OR=43, 95% CI [12.26-151.87].Children living 5Km within a Health facility were 10 times more like to have Measles (95% CI [1.44-69.26] diarrhea and pneumonia were the commonest complications reported after measles 48% and 16% respectively. Among persons interviewed measles vaccine uptake was 36%. Household income, history of recent travel and overcrowding were not statistically signicantly. In conclusion, measles outbreak was confirmed and risk factors included contact with a measles case, lack of vaccination . Reactive vaccination campaign, prompt case management and Health promotion activities were instituted.

1206

TRENDS IN THE TEMPORAL AND SPATIAL DISTRIBUTION OF YELLOW FEVER IN SOUTH AMERICA

Renato P. Souza[1], Adriana Yurika Maeda[1], Terezinha Lisieux Coimbra[1], Selma Petrella[1], Francisco Chiaravalloti Neto[2]

[1]Instituto Adolfo Lutz, São Paulo, Brazil, [2]Faculdade de Saúde Pública, Universidade de São Paulo, São Paulo, Brazil

Yellow fever is a viral infectious disease, endemic in tropical regions of Africa and America. The disease has two transmission patterns, a sylvatic cycle and urban cycle, both leading to clinical manifestations ranging from asymptomatic to mild and severe forms. The sylvatic cycle involves mosquitoes and monkeys. Genetic studies of YFV strains have revealed that strains isolated in South America and Africa are genetically distinct. Nucleotide sequences of envelope region of the genomes of 10 yellow fever virus samples isolated from monkeys, humans or mosquitoes in Brazil between 2008-2010 were determined with the objective of establishing the genotypes and studying the plylogeographical process throughout South America. These fragments were aligned with 60 representative sequences of Yellow Fever, retrieved from GenBank. The Bayesian inference method available in the software BEAST v. 1.6.2 was used in order to analyze the phylogenetic relationship of the strains of this study. Each sequence of the corresponding data set was dated and maximum clade credibility (MCC) tree was generated. The internal nodes were inferred using a Markov Chain Monte Carlo (MCMC) Bayesian approach under a HKY model with a proportion of invariable sites (I), using a relaxed (uncorrelated lognormal) molecular clock. We performed a discrete phylogeographic analysis across 23 localities generating a graphic view of the virus distribution and circulation using SPREAD to plot the geographic coordinates (Latitude and longitude) and GOOGLE EARTH 5.2 to visualization. The obtained MCC Tree suggests a "Source-Sink" pattern of transmission with secondary phylogeographic structure of wave-like transmission as ancient lineages of Yellow Fever are constantly replaced by new ones, and each new introduction causes the seasonal epidemics and epizooties. The discrete phylogeographic analysis suggests that the Amazonian region presents better conditions to maintain viral circulation and diversity and may represent Yellow Fever genetic diversity source.

1207

DEFINING PRODUCT REQUIREMENTS FOR FIELD-DEPLOYABLE YELLOW FEVER TESTS

Roger B. Peck, Jeffrey D. Wellhausen, Molly G. Boettcher, Louise Downing

PATH, Seattle, WA, United States

The fight against yellow fever could benefit from the development of field-deployable tests. Such tests would have immediate utility for diagnosing individual cases and outbreaks as well as contributing to surveillance and control programs. Tests that are currently available for yellow fever are laboratory based, requiring both good infrastructure and trained personnel, and often have a long turnaround time to results. Field tests performed by minimally trained health staff could provide rapid results, allowing an appropriate response to outbreaks and streamlining of laboratory testing for case confirmation. The authors report results of international and country-level stakeholder interviews on the topic of response to outbreaks of yellow fever, case management, and the need for diagnostic testing. The responses have been synthesized to generate use case scenarios identifying specific cases in which a field test would be used. Additionally, target product profiles that include the characteristics required of field tests in order to address these identified use case scenarios have been created from the information collected during the interviews. These use case scenarios and target product profiles will be used to inform the research and development of field-deployable yellow fever tests and their introduction at the country level by operational organizations, leading to accelerated implementation. In parallel, the information will also be used to inform strategies for verification and

validation of such tests; begin to identify commercial viability, including market size, segmentation, and uptake; and introduction at the country level by operational organizations leading to accelerated implementation.

1208

QUESTIONS RAISED BY AN ANALYSIS OF VISCEROTROPIC REACTIONS TO THE YELLOW FEVER VACCINE

Stephen J. Seligman

New York Medical College, Valhalla, NY, United States

The live virus yellow fever vaccine is integral to the control of yellow fever. Previously thought to be the safest of the live virus vaccines, it is now known to cause a rare but frequently serious and often fatal disease termed yellow fever vaccine-associated viscerotropic disease (YEL-AVD). Analysis of known cases has revealed that the vast majority can be assigned to risk groups: males ≥56, women in the prime child-bearing years, individuals thymectomized as treatment for thymoma, infants and children ≤11, women with systemic lupus erythematosis, and brothers with Addison's disease. In addition there was one case each with five other autoimmune diseases and one case whose older sister had died of YEL-AVD. Questions raised by the analysis include: 1) what is an appropriate estimate of the incidence? Although usually stated as 0.3 to 0.4 per 100,000 vaccinees, the data from individual studies vary from none in 2.6 million in Africa to 11.7 per 100,000 in Peru; 2) Do the variations in case fatality rate from 88% in South America to 41% in prospective travelers reflect differences in reporting or differences in vaccine safety (vaccine from South America is a derivative of 17DD and that used elsewhere of 17D-204); 3) Does the occurrence of three cases in young women in Peru reflect an unsuspected ethnic association possibly even related to a case in a young Spanish woman?; 4) Are there underlying genetic immune defects in each of the risk groups and is there a commonality in the defects such as a deficiency in thymic function or a defect in innate immunity; and 5) Does the concentration of fatal cases in women between the ages of 19 and 34 result from factors likely to be present in that age group? Investigation of these questions ranges from the simple ascertainment of the family history (known only in part for one of the young Peruvian women) to sequencing of the human genome. Answers are of concern not only to recommendations for the administration of yellow fever vaccine but also to the safety of vaccines under development that incorporate the yellow fever virus vaccine background.

1209

GENETIC DETERMINANTS ASSOCIATED WITH TICK AND MOSQUITO FLAVIVIRUS COMPETENCE

Joan L. Kenney[1], Michael Anishchenko[1], Ching-I Chen[2], Aaron C. Brault[1]

[1]Centers for Disease Control and Prevention, Fort Collins, CO, United States, [2]University of California Davis, Davis, CA, United States

Flaviviruses are positive-strand RNA viruses that infect more than 100 million people each year. While most flaviviruses exist in an arthropod vector (tick or mosquito)-vertebrate host life cycle, some flaviviruses have adapted to a single host life cycle in either an arthropod or a vertebrate host. The genetic determinants for flavivirus adaptation to a life cycle (dual host or single host) and whether the cycle type specificity constrains the virus's ability to adapt when subjected to new selective pressures has yet to be determined. Understanding the genetic determinants and adaptive constraints these factors impart on these flaviviruses will enhance our ability to design an attenuated, host-restricted, yet replication-competent vaccine candidate. Chimeric constructs between mosquito-vectored West Nile virus (WNV) and the tick-vectored virus deer tick virus (DTV) have been developed to identify the genetic elements that are responsible for host tropism and evaluate the versatility of these determinants in mammalian, tick, and mosquito cell types as well as an *in vivo* models for these systems. In mosquito and Vero cells, WNV parental and DTV-prME/WNV chimeric virus replication profiles did not differ and while the DTV parental and WNV-prME/DTV chimeric virus exhibited similar replication patterns. In ISE6 *Ixodes* tick cells, DTV reached a peak titer of 6.3 log10 PFU/ml, whereas the WNV-prME/DTV chimeric virus only reached a peak of 4.3 log10 PFU/ml. WNV and DTV-prME/WNV viruses were both poorly fit in tick cells, replicating to just above the level of detection. Our current findings indicate that for mosquito cells (C6/36) and mammalian cells (Veros), the nonstructural regions appear to be the dominant determinants of viral phenotype; however, in tick cells the determinants appear to reside in both the structural and nonstructural elements.

1210

EVALUATION OF THE T380R MUTATION IN THE RGD MOTIF OF YELLOW FEVER VIRUS ENVELOPE PROTEIN DOMAIN III IN *AEDES AEGYPTI*

Yan-Jang S. Huang[1], John T. Nuckols[2], Dana L. Vanlandingham[1], Mario Lobigs[3], Stephen Higgs[1]

[1]Biosecurity Research Institute, Kansas State University, Manhattan, KS, United States, [2]Medical Countermeasure Systems, Joint Vaccine Acquisition Program, Fort Detrick, MD, United States, [3]Australian Infectious Diseases Research Centre The University of Queensland, Queensland, Australia

Glycosaminoglycans are ubiquitously expressed negatively charged macromolecules that have been found to support viral entry and dissemination in several arboviruses. The RGD motif is located in the envelope protein domain III (EDIII) of the mosquito-borne flaviviruses. This motif has been found significant in the binding interactions between virions and negatively charged molecules *in vitro* as well as viral dissemination and the associated viral pathogenesis in mammals. However, its importance in viral infection and dissemination in the mosquito vectors remains to be evaluated. Knowledge of this mechanism is critical for understanding the governance of vector competence of the mosquito-borne flaviviruses. As the prototypical flavivirus, the reverse genetics system of yellow fever virus (YFV) provides a unique platform to characterize and evaluate the individual genetic *loci* in the viral genome by the comparison of the virulent Asibi strain, which disseminates and infects mosquitoes, and the live-attenuated 17D vaccine strain, which does not have the capacity to disseminate attributable to mutations generated during the serial passage process. An *in vitro* transcribed viral RNA rescued mutant virus containing the T380R mutation located within the RGD motif in EDIII of YFV 17D strain was evaluated for infection and dissemination capability in *Aedes aegypti* mosquitoes infected per os. Female mosquitoes were collected and dissected on 7, 10 and 14 days post infection. The bodies and secondary tissues of these mosquitoes were homogenized and titrated to assess the efficiencies of viral infection, replication and dissemination. These titrations provided the knowledge to foster the identification of a genetic determinant for the vector competence and viral attenuation of YFV.

1211

PHENOTYPIC CHARACTERIZATION OF YELLOW FEVER VIRUS CONTAINING TWO POINT MUTATIONS IN THE ENVELOPE DOMAIN I AND DOMAIN II

Yan-Jang S. Huang[1], John Nuckols[2], Stephen Higgs[1], Dana Vanlandingham[1]

[1]Biosecurity Research Institute, Kansas State University, Manhattan, KS, United States, [2]Medical Countermeasure Systems Joint Vaccine Acquisition Program, Fort Detrick, MD, United States

The limit infectivity and dissemination of the attenuated yellow fever virus (YFV) 17D vaccine strain in *Aedes aegypti* is attributed to mutations in the envelope protein. The five specific mutations in the envelope protein domain I (EDI) and domain II (EDII) have significantly reduced the infection rate among the engorged mosquitoes at 14 days post infection. Although the flavivirus EDI and EDII domains are not directly involved in host cell receptor binding, their role in supporting structural rearrangement and membrane fusion have been found essential for effective viral entry. Two

point mutations, G52R and T173I, were selected for non-conservative changes in the biochemical properties of amino acid substitutions and their proximities to the molecular hinge regions that accommodate the structural alterations generated during the viral entry. The objective of this study was to identify the genetic *loci* that reduced the viral infectivity of YFV Asibi strain amidst the serial passage process resulting in the attenuated YFV 17D vaccine strain. Mutations were introduced simultaneously and separately into the cDNA infectious clones of YFV to evaluate their infectivity and dissemination in *Ae. aegypti*. Mutant viruses were recovered from *in vitro* transcribed RNA and administered to *Ae. aegypti* per os in a bloodmeal. The viral infectivity, replication and dissemination capacities were evaluated by TCID50 titration of homogenized bodies, heads, wings and legs from mosquitoes collected on 7, 10 and 14 days post infection. Genetic stability of mutant viruses was also determined by nucleotide sequencing.

1212

WEST NILE VIRUS INFECTION INFLUENCES SYMBIOTIC BACTERIAL POPULATION MODULATIONS IN *CULEX PIPIENS*

Greta A. Van Slyke, Alexander T. Ciota, Dylan J. Ehrbar, Laura D. Kramer

Wadsworth Center, Slingerlands, NY, United States

We are beginning to explore the natural microbial community that exists within insect vectors and the effects the mosquito microbiome is eliciting on vector-virus interactions and ultimately the transmission of arboviral pathogens. *Wolbachia,* a natural symbiotic bacterium of many mosquito species including *Culex pipiens*, has been the focus of many studies which have shown repeatedly that this bacterium has an effect on arboviral susceptibility, replication and transmission. It has been reported that *Wolbachia* limits West Nile Virus (WNV) susceptibility in *Cx. quinquefasciactus* and replication in *Aedes aegypti*, similar to studies with dengue virus, chikungunya virus and yellow fever virus, yet these negative correlations seemed to be bacterial and virus-strain dependent, suggesting complex interactions between symbiotic bacteria, host/vector and pathogen strain. Our results demonstrate a positive correlation between relative proportions of *Wolbachia* and WNV viral load in *Cx. pipiens*. Although *Wolbachia* has been the focus of most studies, it is likely that interactions with other bacterial species similarly have the capacity to modulate arboviral infections in mosquitoes. Our results suggest that the *Cx. pipiens* microbiome is being affected by *and* having an effect on WNV susceptibility in our colonized mosquitoes, where shifts in microbial populations are observed upon exposure alone to virus and are not predicted simply by infection status but, by WNV exposure in the absence of detectable infection. Specifically, our data demonstrate that many individual bacterial strains and/or species are associated with viral exposure and effects appear to be strain-specific and reveal novel correlations between the relative amount of Flavobacteria and Enterobacteria with WNV body titer.

1213

DEVELOPMENT OF IMMUNOHISTOCHEMICAL TECHNIQUES FOR DEFINING THE PATHOGENESIS OF WEST NILE VIRUS IN THE BRAIN OF EXPERIMENTALLY INFECTED HORSES

Gretchen H. Delcambre

University of Florida College of Veterinary Medicine, Gainesville, FL, United States

West Nile virus (WNV) induces polioencephalomyelitis and results in the death of one-third of symptomatic horses and a 6% mortality rate in humans. This neurotropic virus elicits an immune response from both infiltrative, peripheral immune cells and resident glial cells. Perivascular cuffing and gliosis are commonly found pathologic changes in the basal ganglia, thalamus, brainstem, and ventral horn of the spinal cord in both species. To date, descriptive analysis of the inflammatory response in WNV infected brains is mostly of experimental disease in rodent models and not in the naturally occurring, outbred equine host. This study quantifies the cell populations found in the diencephalon and metencephalon of horses experimentally infected with WNV via intrathecal inoculation. Based on target antigen location and antibody specificity, immunohistochemical protocols were developed for the identification of resident and peripheral cells in the brain in formalin fixed paraffin embedded and snap-frozen, fresh tissues. Monoclonal and polyclonal antibodies, developed from a variety of species including human, swine, and equine, were used for identification of CD3+,CD4+, CD8+, and B lymphocytes, macrophages, astrocytes, microglia, and neurons. Early results demonstrate that CD3+ T-lymphocytes are the dominant infiltrative population with few macrophages/neutrophils and scarce CD 79+ B-cells. CD4+ and CD8+ populations are currently under investigation. Microgliosis was significantly higher in infected tissues as compared to control tissues, whereas astrocyte counts were not significantly different. Highly variable staining of neurons in both infected and non-infected horses was noted and is undergoing additional investigation. This study will provide new information in understanding the basic pathophysiology of WNV in the naturally occurring host and may bring new light to the role of uncommonly studied cell types like microglia.

1214

WOLBACHIA DOES NOT INHIBIT WEST NILE VIRUS (WNV) IN THE MOSQUITO *CULEX TARSALIS*

Brittany L. Dodson[1], Grant L. Hughes[1], Oluwatobi Paul[2], Amy C. Matacchiero[3], Laura D. Kramer[3], Jason L. Rasgon[1]

[1]Pennsylvania State University, University Park, PA, United States, [2]University of Maryland Baltimore County, Baltimore, MD, United States, [3]Arbovirus Laboratories, Wadsworth Center, New York State Department of Health, Slingerlands, NY, United States

Novel strategies are required to control mosquitoes and the pathogens they transmit. One attractive approach involves the maternally inherited endosymbiotic bacterium, *Wolbachia*. After artificial infection with *Wolbachia*, many mosquitoes become refractory to infection and transmission with diverse pathogens. By manipulating arthropod reproduction, *Wolbachia* can spread throughout the host population, replacing the natural vector population with one that cannot maintain pathogen transmission cycles. We evaluated the effects of *Wolbachia* (*w*AlbB strain) on infection, dissemination and transmission of West Nile virus (WNV) in the mosquito *Culex tarsalis*. After inoculation into adult female mosquitoes, *Wolbachia* reached high titers and disseminated widely to numerous tissues including the head, proboscis, ovarian follicles, thoracic flight muscles, and fat body. Contrary to other systems, *Wolbachia* did not inhibit WNV in this mosquito. Rather, in the majority of replicates, WNV infection rate was significantly higher in *Wolbachia*-infected mosquitoes compared to controls. This is the first observation of *Wolbachia*-induced enhancement of a human pathogen in mosquitoes, suggesting that caution should be applied before using *Wolbachia* as part of a vector-borne disease control program.

1215

EVOLUTION OF WEST NILE FEVER IN EUROPE: CLOSE MONITORING OF THE DISEASE SINCE 2011

Laurence Marrama Rakotoarivony, Carlijn Bogaardt, Eve Robinson, Virginia Estevez, Pasi Penttinen, Josep Jansa, Denis Coulombier, Herve Zeller

ECDC, Stockholm, Sweden

West Nile fever is a viral disease transmitted by mosquitoes that affects birds, equids and humans. Humans usually present asymptomatic infection or mild disease but neuro-invasive infection occurs. Crucially the virus can be transmitted through blood transfusion or organ transplants. The disease has been known in Europe for more than 50 years but it particularly re-emerged in Romania in 1996. Since 2004, two lineages have been co-circulating in the European Union. And since 2010, a geographical

expansion has been observed. To appraise the evolution of the disease, ECDC monitors human cases in Europe and neighbouring countries using epidemic intelligence methods. Since 2011, information concerning human cases from 63 countries of the WHO European region and the Mediterranean basin is collected through the West Nile mapping tool and publicly displayed on ECDC website, on a weekly basis, during the transmission season (June to November). Comparison of results obtained in 2011 and 2012 shows that, in 2012, the first cases were reported much earlier, the extension of the affected zone was broader and the density of areas reporting cases was higher. Hence, the total number of cases was higher in 2012 in the European Union (237 vs. 124) and neighbouring countries (670 vs. 203). In addition, several countries reported cases for the first time. Comparison of the geographic extension since 2010 shows that new areas were affected each year. In the evolution of the disease since 2010, the respective role of enhanced surveillance and change in the epidemiology of West Nile fever is discussed. On one hand, surveillance activities have been reinforced, i.e. awareness of health professionals is higher, special/active surveillance activities have been implemented. On the other hand, the epidemiology of West Nile fever has also changed, two lineages co-circulate in many countries and new strains are detected. In addition, some new questions are raised, e. g. the role of multiple introductions of the virus, the relations between animal and human cases, the impact of the climate.

1216

GENETIC ANALYSIS OF WEST NILE VIRUS HUMAN ISOLATES FROM THE U.S. SHOWS STEADILY INCREASING VARIABILITY FROM 2002 - 2012

Caren Chancey, Andriyan Grinev, German Añez, Daniel A. Heisey, Maria Rios

CBER/Food and Drug Administration, Bethesda, MD, United States

Since introduction into the U.S. in 1999, West Nile virus (WNV) has become endemic, causing annual outbreaks for 14 consecutive years. The rapid spread of WNV and pattern of recurring outbreaks in the US differs from the sporadic outbreaks previously observed in the old world, suggesting a potential role for viral adaptation to domestic vectors and hosts. Viral adaptation through genetic mutations has the potential to alter viral growth characteristics and virulence. Most (80%) human infections with WNV are asymptomatic; of the symptomatic cases, <1% are neuroinvasive infections (1:150 to 1:350 total infections) which have a high mortality rate (14%). Over the past 14 years WNV has caused ~37,000 human cases of serious illnesses including 15,975 neuroinvasive cases and 1,505 deaths reported to the CDC, with intense outbreaks of human cases in 2002 (4,156 cases) 2003 (9,862), 2006 (4,269) and 2012 (5,387). We investigated the degree of genetic variation in human infection by sequencing 78 (47 full and 31 partial) WNV isolates produced from human plasma, obtained from different geographical locations of the U.S. from 2002-2012. Compared to the sequence of the ancestor strain WNV-NY99, results showed increasing genetic variability over time including deletions and insertions in the 3'UTR. Most mutations were silent transitions, and the number of nucleotide mutations ranged from 20 to 81 resulting in 3 to 17 amino acid substitutions. Our study shows that over 14 years in the U.S., the WNV genome varied at a slow but steady rate when compared with WNV-NY99; increasing from 0.18% diversity in 2002 to 0.54% in 2009 and 0.65% in 2012. The mean nucleotide substitution rate for our WNV isolates through 2011 was 5.06×10^{-4} substitutions/site/year. Genetic surveillance is essential for public health since mutations could potentially affect viral pathogenesis, decrease performance of diagnostic assays, and negatively impact efficacy of vaccines and development of specific therapies.

1217

DEVELOPMENT OF A MICROARRAY-BASED ASSAY TO PERFORM MOLECULAR EPIDEMIOLOGICAL SURVEILLANCE OF WEST NILE VIRUS

Andriyan Grinev, Caren Chancey, Evgeniya Volkova, Vladimir Chizhikov, Maria Rios

CBER/Food and Drug Administration, Bethesda, MD, United States

Since its first detection in the U.S. in 1999, West Nile virus (WNV) has become endemic in the Western Hemisphere. WNV is primarily transmitted by mosquito bites, but can also be transmitted by blood transfusion. WNV is estimated to have infected ~4 million humans in the US causing >36,000 cases of severe disease and 1,505 deaths reported to CDC. The speed of WNV's spread raised great concern and prompted a detailed investigation of the genetic evolution of WNV in search of causes for its rapid adaptability. Of concern is the potential for mutations in the WNV genome to affect the performance of diagnostic and screening assays, viral pathogenesis and therapeutic approaches. Thus, the detection of variants that may appear in the course of WNV outbreaks is extremely important and can only be achieved by the genomic characterization of new WNV isolates in a timely fashion. To address this need, we have developed a microarray-based assay capable of direct analysis of genetic variation in field specimens. The assay detects mutations by hybridizing biotinylated samples to oligoprobes covalently immobilized on slides surface covering the target region of the WNV genome with subsequent silver staining. Assay validation was performed using previously sequenced WNV isolates from the US, 2002-2012. The new assay detected unambiguously all mutations previously identified by traditional sequencing analysis. In addition the new assay eliminated the need for viral isolation by tissue culture, because viral RNA is isolated directly from plasma using magnetic beads loaded with specific oligoprobes. The identified mutants can be further sequenced using classical Sanger or next generation sequencing methods to determine position and character of mutations. The use of the described microarray for an initial screening of WNV isolates can notably reduce the time of analyses of circulating genetic variants and sequencing related expenses, and can be used as a valuable tool for rapid epidemiological surveillance of WNV during annual outbreaks.

1218

CHARACTERIZATION OF WEST NILE VIRUS-INDUCED MEMBRANE STRUCTURES REVEALS A NOVEL ROLE FOR NS1 PROTEIN IN INDUCTION OF VESICLE PACKETS FOR VIRAL RNA SYNTHESIS

Alanna Tseng[1], Pakieli H. Kaufusi[2], James F. Kelley[2], Vivek R. Nerurkar[2]

[1]University of Hawaii at Manoa, Honolulu, HI, United States, [2]John A. Burns School of Medicine, University of Hawaii, Honolulu, HI, United States

West Nile Virus (WNV) modifies intracellular membranes of the host cell, resulting in the formation of clusters of vesicle packets (VP) to establish its sites of replication. Although the role of VP for viral RNA synthesis is well documented, the WNV protein(s) responsible for the induction of the VP has not yet been identified. In this study, we describe a novel role for NS1 in the induction of these VP structures during WNV infection. We infected HEK293T cells with the WNV New York 1999 strain (NY99) and analyzed the localization of the NS1 protein in VP structures over time using immunofluorescence (IF) assay. WNV replication was determined using qRT-PCR and plaque assays, and NS1 protein expression was verified using Western Blot. To demonstrate the induction of VP with only NS1 protein, we synthesized cDNA from WNV NY99 RNA using reverse transcriptase and amplified the entire NS1 gene, including the endogenous signal sequence from the WNV envelope structural protein, by high-fidelity PCR. To visualize expressed NS1 in transfected cells, green fluorescent protein (GFP) or V5 epitope harbored in the expression vector was fused to the C-terminal end of the NS1 gene. Plasmids were transformed into

chemically competent *E. coli* (DH5α) cells and were isolated, purified and sequenced. The resulting plasmids were used for expression of NS1 protein in transfected HEK293T cells. The VP structures induced by only NS1 were analyzed using transmission electron microscopy (TEM). Using high-resolution confocal microscopy, NS1 protein was found to localize to VP, which appeared as fluorescent particles (FPs) scattered in the cytoplasm along with viral replicating RNA. Toward the end of the eclipse phase of infection, aggregation of FPs at the perinuclear region coincided with increases in viral RNA copies and virion production. TEM data using NS1 transfected cells revealed that the NS1-induced membrane structures were similar to VP structures observed in infected cells. NS1 has been implicated in the viral RNA replication and results from this study suggest that NS1 plays a role in VP induction. Studies are underway to explore the sequential events in VP biogenesis, and determine whether any host proteins are involved in VP formation and expansion.

1219

RARE NEUROLOGIC DISORDERS IN HIV POSITIVE CHILDREN TREATED WITH LONGTIME HAART

Andrea Shahum[1], George Benca[2], Julia Vujcikova[2], Veronika Sladeckova[1], Lenka Rabarova[1], Jaroslava Sokolova[3], Nada Kulkova[3], Vladimir Krcmery[1]

[1]St. Maximilian Kolbe Clinic, SEU Tropical Program, House of Family, Phnom Penh, Cambodia, [2]St. Elizabeth University of Health and Social Sciences, Bratislava, Slovakia, [3]Department of Laboratory Medicine, School of Health Care and Social Work, Trnava University, Trnava, Slovakia

Neurological manifestation of AIDS is of at least different etiology. The aim of this short communication is to describe neurologic disorders due to either HIV-related opportunistic coinfection or drug related toxicities in children with AIDS from Cambodia treated 10 years with highly active antiretroviral treatment (HAART). One hundred thirty seven (137) children (age 2-19 years) treated with HAART since 2003 (6-10 years follow up) have been analyzed concerning opportunistic infection, other coinfection, renal, hematologic parameters, X- chest ray, received of antiretrovirals (HAART), antituberculotics and antibiotics. We recorded prospectively all data since onset of HAART in 2003 - 2005 up to December 2012. Incidence of drug related neuritis due to stavudine (HAART) was less than 1% and have due to anti-TB agents accompanying HAART in HIV and tuberculosis coinfected patients. Coinfections with neurotropic viruses were higher: 1 case CMV retinitis, 18 herpes zoster neuritis and 37 generalized HZV infections (varicella) after onset HAART in children were recorded. All children responded well to antivirotic therapy with ganciclovir (CMV), vanciclovir or acyclovir (HZV) in combination with first line HAART. Neurologic disorders in children with HAART are less frequent than in adults. Antiretovirals and antituberculotic seems to be safe and less hemotoxic as reported in children then adults. Coinfections with neurotropic viruses were higher. All children responded well to antivirotic therapy with ganciclovir (CMV), vanciclovir or acyclovir (HZV) in combination with first line HAART.

1220

CHALLENGES OF FINDING HIV PATIENTS WHO HAVE DROPPED OUT OF CARE AFTER DIAGNOSIS AT AN HIV REFERENCE SERVICE IN LIMA, PERU

Jorge R. Paz[1], Cesar Ugarte-Gil[1], Aaron M. Kipp[2], Carla V. Valenzuela[3], Juan Echevarria[4], Sten Vermund[2], Eduardo Gotuzzo[4]

[1]Instituto de Medicina Tropical Alexander von Humboldt, Lima, Peru, [2]Vanderbilt Institute for Global Health, Nashville, TN, United States, [3]Vanderbilt University School of Medicine, Nashville, TN, United States, [4]Hospital Nacional Cayetano Heredia, Lima, Peru

In low-resource settings, HIV programs have difficulties finding patients who have dropped out of care, resulting in increased morbidity and HIV transmission. Stigma, discrimination and access to the clinic are main reasons for this problem. However, there is not information available about the status of patients who dropped out in Lima (Peru) to identify these patients and prepare an adequate intervention. This study has the aim to determine the status of HIV patients currently out of care at the HIV Clinic at Hospital Nacional Cayetano Heredia in Lima, Peru since HAART program was established. Among 3085 enrolled patients between 2004-2010, we identified 753 adult patients whose last medical care visit at the HIV Service occurred at least 12 months before March 2011. Patients with phone numbers were called first and home visits were attempted for patients who could not be reached by phone. This contact data was registered at enrollment into HIV Clinic. No exclusion criteria were used. The mean age among the 753 patients was 37.97 (SD: 11.5) and 475 (63.1%) were male. The median CD4 at enrollment was 246.5 (IQR: 354). 134 (17.8%) were located, 29.9% (40/134) of them were dead. 434 (57.6%) were not located for the following reasons: false address (18.9%), didn't live in the address provided (19.1%), hard to find the address (13.8%), the patient move and don't provide new address (17.3%) and non phone answer (30.9%). 185 (24.6%) were not approached for having incomplete data (31.4%) or for living outside hospital`s area of influence (68.6%). Non-located patients were not different in sex (64.94% vs. 54.47% males), age (38.02 years vs. 37.7 years; p-value 0.79) and CD count (238.5 vs. 257; p-value: 0.58) compared with located patients. Nearly 82% of HIV patients who dropped out of care cannot be found, and these patients are not different than located patients. Improving retention in care requires addressing the reported barriers and implementing a follow-up system to verify and update patient contact information until they can be returned to care.

1221

METHODOLOGICAL FLAWS IN THE HIV-STI TRIALS

Larry Sawers[1], Eileen Stillwaggon[2]

[1]American University, Washington, DC, United States, [2]Gettysburg College, Gettysburg, PA, United States

Substantial evidence indicates that sexually transmitted infections (STIs) promote HIV transmission by producing genital ulcers, inflammation, and viral shedding. The burden of untreated STIs is far higher in sub-Saharan Africa (SSA) than in any other region. Ten randomized controlled trials in SSA examined effects of STI control on HIV incidence. Only the first trial (Mwanza) produced statistically significant results. Consequently, support for STI treatment for HIV prevention has faded. The 9 post-Mwanza trials suffer from insufficient exposure contrast because ethical considerations require extensive STI treatments for controls, leading to small differences in STI outcomes between arms. The trials tested alternative treatments of bacterial or viral STIs, not both, and were thus subject to confounding from STIs not considered in the trial design. Substantial treatments for controls affected sexual behavior and STI outcomes between arms. No trials examined genital infections other than STIs that could enhance HIV transmission or acquisition. Thus none of the trials addressed potential effect modification from biological interactions in genital microbial communities. None of the trials considered genital ulceration and inflammation from non-sexually transmitted pathogens, most importantly Schistosomiasis hematobium, which is highly prevalent in SSA. None considered ulcers infected with streptococci, staphylococci, or fungi. Treating one genital infection may have little effect on HIV incidence when other infections are untreated. All of the trials that estimated pre-trial HIV incidence found statistically significant decreases in HIV incidence among treatment subjects and controls taken together, suggesting that STI control programs can be effective in reducing HIV. Flaws in the design of the post-Mwanza trials render them unable to inform HIV-prevention policy. Given abundant evidence that STIs and other genital infections promote HIV, STI and other treatment should be considered an important method for reducing HIV incidence in SSA and elsewhere.

1222

FOLLOW-UP OF HIV-EPTB COINFECTION BY ULTRASOUND

Maria Teresa Giordani[1], Enrico Brunetti[2], Clara Stecca[1], Paolo Benedetti[1], Sam Goblirsch[3], Tom Heller[4]

[1]Infectious and Tropical Diseases Department, san Bortolo Hospital, Vicenza, Italy, [2]Department of Infectious Diseases, IRCCS San Matteo Hospital Foundation, University of Pavia, Pavia, Italy, [3]Department of Emergency Medicine, Winona Health, Winona, MN, United States, [4]Department of Medicine, Klinikum Muenchen-Perlach, Munich, Germany

Extrapulmonary and disseminated TB coinfection in HIV patients mainly affects individuals in Sub-Saharan Africa but also immigrants living in Europe. The disease is difficult to diagnose and is most effectively treated when diagnosed early. Ultrasound can detect suggestive findings such as enlarged abdominal lymph nodes, pleural and peritoneal effusions and focal lesions in the liver and spleen. In some resource-poor settings these findings are relied upon to start empiric TB treatment. However, longitudinal changes of this ultrasound (US) pattern during TB treatment have not been investigated. In a retrospective study, 25 patients with culture confirmed HIV-EPTB co-infection were diagnosed and treated between 2005 and 2011 in our Hospital. Seventeen patients (mean age 36.4 , range 24-57, M/F 1.83 , 11 of them (64.7 %) african immigrants , mean CD4 149.11(range 5-576) had follow-up US (FASH protocol) at 1, 3, 6 and 12 months. In 7 patients (41,17 %) after 1 month of treatment, the FASH findings had completely disappeared, in 2 patients they had partially disappeared; all 9 patients successfully completed TB therapy. In the remaining 8 patients the US examination performed at 1 month showed an increased size of the previously detected lesions and/or the onset of new lesions: among them, three patients died during the follow-up (one while treating TB coinfection and two after completing TB treatment for AIDS-related conditions), one patient had MDR - TB and was lost to follow-up after different courses of therapy, three patients had a relapse, likely related to poor compliance; in one patient with a prolonged course of the infection the lesions disappeared only after two years of treatment. In conclusion, our experience suggests that early regression (after 1 month of therapy) of HIV-EPTB US signs is a good prognostic index for treatment in patients with HIV-EPTB. US protocols for HIV-EPTB co-infection should be validated in prospective studies as a potentially useful tool for the follow up of HIV-EPTB.

1223

CLINICAL AND LABORATORY CHARACTERISTICS OF DENGUE AND HIV CO-INFECTION: A MATCHED CASE-CONTROL STUDY

Junxiong, Vincent Pang[1], Tun L Thein[2], Yee-SIn Leo[3], David C. Lye[3]

[1]Saw Swee Hock School of Public Health, National University of Singapore, Singapore, Singapore, [2]Communicable Disease Center, Tan Tock Seng Hospital, Singapore, Singapore, [3]Department of Infectious Diseases, Tan Tock Seng Hospital, Singapore, Singapore

Coinfection of dengue virus and human immunodeficiency virus (HIV) has been reported as case series with patients generally having mild manifestations even though they are immunocompromised. However, there is a lack of reported epidemiological study to support this hypothesis. In this retrospective hospital-based 1:4 matched case-control study, the clinical and laboratory profiles of ten Chinese HIV-infected dengue patients (cases) were compared with 40 Chinese HIV-noninfected dengue patients (controls), matched for age, gender, dengue diagnosis method, year of dengue presentation and hospitalization status. Univariate and multivariate conditional logistic regression were performed. Seven out of 10 cases had AIDS at the point of dengue presentation. The median period from HIV positive diagnosis to dengue presentation was 36.5 months with median CD4 counts of about 123 cells/mm3 six months before and after dengue presentation. A smaller proportion of cases (10%) was classified as DHF/DSS as compared to controls (52.5%;P=0.046). In contrast, a higher proportion of cases (50%) were classified as having severe dengue compared to controls (20%;P=0.05). The median fever duration was shorter in cases (3days) compared to controls (4days; P=0.032). However, the median hospitalization duration was longer in cases (10.5days) compared to controls (5days;P=0.054). There is no significant difference in supportive treatments even though the occurrence of tachycardia and shock were higher in cases (70%;90%) compared to controls (20%;35%) during hospitalisation. During presentation and hospitalisation, maximum pulse rate [adjusted conditional odds ratio (ACOR)= 1.31;95% confidence interval(CI)=1.02-1.25; ACOR=1.11;95%CI=1.01-1.22) and esinophils level (ACOR=2.82;95%CI=1.06-7.51; ACOR=1.90;95%CI=1.17-3.09) were positively associated with coinfected patients. HIV-infected dengue patients may clinically present as mild as HIV-noninfected dengue patients during presentation, but may not throughout the hospitalisation. Close observation and supportive treatments for HIV-infected patients coinfected with dengue remain critical.

1224

CROSS-CULTURAL EXEMPLARS ON HIV/AIDS RISK AND RESILIENCE AMONG YOUTH: THE PERSPECTIVE OF CHILD AND ADOLESCENTS FROM DIFFERENT BACKGROUNDS

Chinedu O. Oraka, Samuel C. Ani

College of Health Sciences, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria

This study was undertaken to examine evidence available by considering the exemplars or indicators of categories in risk and resilience among youth as well as child abuse and neglect as it directly exposes them to risks of HIV/AIDS infection. Child abuse has become an international concern and has been discussed extensively in developed countries and regions, example of which is now being translated to the developing nations. A retrospective cohort study of children and youths that visited the clinics both in Nigeria and Malaysia by accessing the hospital medical records and data was identified from linked de-identified population level data. Results were analysed on the patterns and views as well as trends in prevalence of assault, maltreatment, risk and resilience, and risky sexual practices as a result leading to HIV/AIDS/STDs; which were further investigated. It is pertinent to note that - Youth and children's disclosure of abuse is often affected by the culture in which they live, like filial piety and loyalty to parents. Some of the views expressed by children, however, are very much akin to those of adults, such as the factors they would consider in deciding whether a case is child abuse or not. Youths do not have a homogeneous view on issues about the risk and resilience existing in child abuse and neglect, and their awareness and sensitivity to different kinds of child abuse are also different, also leading to varying levels of information about HIV/AIDS and sexual education. In conclusion, there has been steady increase in the prevalence of assault and maltreatment. In order to continue to develop appropriate services and policies for vulnerable youth, it is necessary to continue definitional clarity for research in child maltreatment, in tandem with parental and child characteristics which can provide one source of evidence-basis to meaningful child protection case classifications.

1225

CONTRIBUTION OF XPERT MTB/RIF ASSAY IN THE DIAGNOSIS OF TUBERCULOSIS AMONG PATIENTS LIVING WITH HIV IN DAKAR, SENEGAL

Awa Ba-Diallo, **Sidiya Mbodj**, Mame Yacine Fall-Niang, Awa Ndiaye-Diawara, Habsa Diagne-Samb, Aissatou Gaye-Diallo, Souleymane Mboup

Hopital A. le Dantec, Dakar, Senegal

Lack of a rapid, sensitive, and specific diagnostic test for tuberculosis (TB), especially in resource poor countries, greatly complicates TB control worldwide. A new diagnostic method based on real time PCR offers an advantage compared to microscopy, which has low sensitivity, and culture,

which has a long turnaround time. We performed a comparative study of diagnostic methods used in a TB endemic region on 128 patients with suspected TB. Sputum samples were analyzed by the Xpert MTB/RIF assay and results were compared to smear microscopy and culture. The study population of 128 patients aged between 18 to 74 included 96 men (75%) and 32 women (25%), of whom 52 patients (40%) tested HIV positive. 32 out of 35 smear and culture positive patients were detected positive by Xpert MTB/RIF assay resulting in 91.4% sensitivity in this group. Only 12 out of 20 patients with a negative smear and positive culture were positive by the assay. Among patients with negative smear and culture 6 out of 65 sputa were detected positive by the assay (9.2%), with one invalid result. All the six patients were HIV positive and naïve to antiretroviral treatment. The time to diagnosis was 2 hours for GeneXpert positive results versus one day for microscopy and 21days for culture. The GeneXpert assay performed well in our setting, with increased sensitivity over microscopy and short turnaround time. The GeneXpert positive sputa that were culture negative were likely true positives that were missed in culture. This comparison of methods available in Dakar shows the need for wider implementation of this new diagnostic test for tuberculosis infection, especially for improved care for people living with HIV.

1226

DRUG METABOLISM AND TRANSPORTER-BASED RATIONALE GUIDES REFORMULATION STRATEGIES TO REDUCE TREATMENT COSTS WITH TDF IN HIV/AIDS

Stephen A. Wring[1], Melynda Watkins[2], Ryan Randolph[1], Seong Hee Park[1], Sara Schock[1], Robin Parham[1], Paul Domanico[2]

[1]SCYNEXIS Inc., Research Triangle Park, NC, United States, [2]Clinton Health Access Initiative, Boston, MA, United States

Tenofovir disoproxil fumarate (TDF) is a bisphosphonate ester pro-drug of tenofovir (TNF) designed to improve oral bioavailability. Nevertheless, bioavailability in humans remains below 30%. Improvements in bioavailability may allow a reduced dose and lead to significant cost savings in generic markets. TDF is subject to a two-step hydrolysis to first the mono-phosphonate ester (TNF-ME) and then to the active TNF. To prioritize reformulation options, drug metabolism and efflux transport were studied to identify targets that might increase the fraction absorbed (f_a). These included stability in simulated gastric (SGF) and intestinal (FaSSIF) fluids +/- pancreatic enzymes, restrictive absorptive permeability, efflux transport by P-glycoprotein (Pgp), and hydrolysis by intra-luminal and enterocytic esterases. TDF and TNF-ME were stable ($T_{½}$ >360 min at 37°C) in SGF and FaSSIF indicating protection from various pH conditions was unwarranted. However, both were unstable ($T_{½}$ <7.5 and 83 min, for TDF and TNF-ME, respectively) in FaSSIF supplemented with physiological levels of pancreatic enzymes. Permeability across MDCK-MDR1 cells demonstrated that TDF, but not TNF-ME or TNF was a substrate for Pgp efflux. Intrinsic permeability for TDF was modest (P_{appA-B}, 23 nm/s) but consistent with oral delivery. Permeability for TNF-ME and TNF was low (P_{appA-B}, <5nm/s) indicating TDF is preferred. Transport and metabolism studies in Caco2 monolayers demonstrated that esterase inhibition by excipients including propyl paraben (PP) increased f_a 8.5 fold (15.3% and 1.8% of dose delivered with PP or in control, respectively). Inhibition of Pgp by d-alpha tocopheryl polyethylene glycol 1000 succinate (TPGS) improved f_a 2 fold (3.5% of dose delivered). Combined esterase and Pgp inhibition increased f_a 10.9 fold (19.7% of dose delivered). In conclusion, *in vitro* studies suggest TNF bioavailability might be significantly improved by co-formulating TDF with excipients that inhibit Pgp efflux and esterase hydrolysis. Bioavailability studies are underway in dogs to evaluate this hypothesis.

1227

PREVALENCE AND BIO BEHAVIORAL CORRELATES OF CARCINOGENIC HPV INFECTION AMONG HIV INFECTED MEN WHO HAVE SEX WITH MEN (MSM) IN LIMA, PERU

Manuel Villaran[1], David Iglesias[1], Jorge Sanchez[1], Nilda Gadea[2], Silvia Montano[2], Joseph Zunt[3]

[1]Asociación Civil Impacta Salud y Educación, Lima, Peru, [2]U.S. Naval Medical Research Unit - 6, Lima, Peru, [3]University of Washington, Seattle, WA, United States

Individuals practicing unprotected anal intercourse are at high-risk for HPV infection and other STI. Currently, fifteen different types of HPV have been classified as of high-risk based in their carcinogenic potential, including anal cancer. Furthermore, STI may have an impact on HPV transmission and acquisition.In 2012 a bio behavioral survey was conducted among 152 adult HIV-positive MSM in Lima, Peru to assess the prevalence and correlates of carcinogenic HPV infection. Subjects underwent a structured behavioral interview and serology testing for HTLV and syphilis, urine testing for *Chlamydia trachomaties* and *Neisseria gonorrhoeae*; and rectal swabbing for HPV DNA testing by PCR. All participants considered themselves either "gay" (75.7%) or bisexual (24.3%); 50.9% reported having a versatile role in bed. The mean time from HIV diagnosis to enrollment was 4.4 years (SD 3.9 years); 78.3% were receiving HAART at the time of enrollment. Mean CD4+ cell count was 437.4 cells/mm^3 (SD 193.5). Eighty participants (52.6%) reported symptoms of STIs in the preceding six months. One hundred forty-seven participants (96.7%) had HPV infection; 90.5% of them with one or more carcinogenic type. The most prevalent types were 6, 16, and 58. The prevalence of urethral chlamydia (2.6%) and gonorrhea (0.7%) were very low. Syphilis seroreactivity was 26.3%. Factors associated with presence of carcinogenic types of HPV were ever having receptive anal intercourse (OR=10.17; 95% C.I. 2.12 - 52.61), having a versatile (OR=5.91; 95% C.I.1.36 - 25.63) or exclusively receptive role in bed (OR= 9.50; 95% C.I. 1.07 - 84.09). Given the high prevalence of carcinogenic types of HPV in the target population, different strategies are needed to improve compliance towards safer sex, including male adolescent HPV vaccination regardless HIV status.

1228

ARVDS ADHERENCE: PATIENTS SELF REPORTS, CD4 COUNTS VS ARVDS BLOOD CONCENTRATIONS

Akinwumi A. Akinyede, Alade Akintonwa, Charles Okany, Olufunsho Awodele, Sunday Olayemi

College of Medicine, University of Lagos, Lagos, Nigeria

Quite a number of researches and publications have addressed HIV infected patents' adherence to prescribed antiretroviral drugs (ARVDs). Adherence to ARVDs ensures adequate blood levels of these drugs with resultant control of HIV disease state and reduction of infectivity. In the regular clinical setting especially those found in resource- limited regions typified by Nigeria, patient self-reports, CD4 counts, viral load, pill counts and pharmacy dispensing data have been used to monitor adherence, however, all have their limitations and may not give an indication of ARVDs absorption. This is against the back drop that the ultimate concern in therapeutics is the knowledge of blood concentrations of ARVDs achieved. Venous blood samples of 10 consecutive HIV infected patients on Lamivudine(L), Zidovudine(Z) and Nevirapine(N) combination for at least 2 months were collected after they consented to be enrolled in the study. Eight(8) of the patients had reported 100% adherence, while 2 of them skipped a dose each 2 weeks prior to the test. These samples were assayed for concentrations of LZN and CD4 count using HPLC Adilent 1200 series and Partec Flow Cytometer. The blood concentrations of L varied from 4.411-17.382 µg/ml (C pss= 1.6µg/ml); Z was 0.2040-0.3825µg/ml (Cmin/Cmax=0.02/2.29 µg/ml); N varied from 1.683-9.944 µg/ml(Cmin/Cmax=3.73/5.74 µg/ml). CD4 counts varied from 227-705 cells/ µL. The patients' reports of adherence to medication was proved right by the observed blood concentrations of the ARVDs which in the case of L was

above Cpss, The concentration of Z was also suggestive of same since it was above Cmin. Though, concentration of N was less than Cmin in some patients, gross analysis also suggest drug adherence. CD4 counts appeared to indicate adherence to ARVDs since the values got correlate with that expected among these patients. Patients self- reports and CD4 counts appear to be useful indicators of ARVDs adherence among this group of patients.

1229

NOVEL TENOFOVIR DISOPROXYL FUMARATE (TDF) FORMULATION TO INCREASE BIOAVAILABILITY FOR TREATMENT OF HIV/AIDS IN EMERGING MARKETS

Melynda Watkins, Paul Domanico, Joanna Tang, David Ripin

Clinton Health Access Initiative, Boston, MA, United States

In the context of funding constraints and the need for continued treatment scale-up for HIV, pursuing interventions with a high return on investment and potential to produce significant global savings is critical. The Clinton Health Access Initiative (CHAI) has initiated a program to develop a novel, lower-dose formulation of TDF. This program will determine whether a lower dose alternative to Viread® will provide the same pharmacokinetics in a novel formulation targeted to increase bioavailability of the active ingredient. In collaboration with major stakeholders during the Conference on Antiretroviral Dose Optimization (CADO) in June 2010, and with input from a range of key experts, including process and formulation chemists, clinical pharmacologists and researchers, ARVs were ranked with the goal of reducing the cost while maintaining the effectiveness of current ARV regimens and to consider additional opportunities for optimizing treatment through manufacturing, formulation and/or dosage forms for delivery in resource limited settings. Reformulation of TDF was identified as the lead candidate to pursue. As TDF reformulation represents a novel approach to optimization, establishing proof of concept (POC) could result in significant contribution to the scientific community guiding similar research efforts for other products. R&D efforts thus far have resulted in more than twenty prototype formulations that have been screened in rat and dog studies. Currently, additional R&D is being conducted in the dog model to identify lead formulations to move into clinical studies. It is anticipated that straightforward, Phase I bioequivalence studies will be required for approval and acceptance of the novel TDF formulation or other reformulated products going forward. A significant effort focused on proactive engagement with regulators, normative bodies, and local opinion leaders is included to ensure that the technology and products being developed will be accepted and adopted in the marketplace in a timely manner. This roll-out platform can also be leveraged to support the uptake of optimized products in the future.

1230

NON-COMPLIANCE DURING MASS AZITHROMYCIN DISTRIBUTION FOR TRACHOMA IN THE GAMBIA

Tansy Edwards[1], Elizabeth Allen[1], Emma Harding-Esch[1], Ansumana Sillah[2], John Hart[1], David Mabey[1], Robin Bailey[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]National Eye Care Programme, Gambian Department of State for Health and Social Welfare, Banjul, Gambia

Untreated individuals in mass treatment programs for infectious diseases can limit the beneficial effects to the community by maintaining a reservoir of infection. Treatment receipt was recorded against the community census during three mass treatment rounds (baseline, year one, year two) as part of a cluster randomised trial, in 48 communities in four districts in The Gambia. Factors associated with non-compliance were investigated in 1-9 year olds using random effects logistic regression. Two types of non-compliers were identified; present during the treatment team visit but not treated (PNT) and eligible for treatment but absent during the visit (EBA). At baseline 99 (1.0%) of 9790 children were PNT and 505 (5.2%) EBA. Non-complier types differed by district (p=0.001) and household size (p<0.001). PNTs were generally from small households in medium to large EA within close proximity to water and with no TF diagnosis in a 0-5 year old in the household prior to treatment at baseline. Regression analyses compared PNTs and EBAs to treated children separately. At the year one and year two treatment rounds, being PNT was more likely for children previously PNT. At baseline, odds of being EBA were higher for children under six years old and for children who had not been examined for trachoma prior to treatment. EBA status was also associated with household level factors. Increased odds of being EBA at year one were seen for EBAs at baseline (OR=4.01; 95% CI:2.43-6.62). Increased odds of being EBA at year two was also noted for baseline EBAs and year one EBAs (OR=1.80; 1.10-2.96 and OR=1.57; 1.17-2.11 respectively). Non-compliance in The Gambia was low overall suggesting positive attitudes towards community-wide treatment. Results showing associations with household level measures and persistent non-compliance lends support to the hypothesis of household level clustering of, and possibly decision making regarding, compliance. Sensitisation of all household heads within communities ahead of treatment team visits could improve compliance amongst the target population.

1231

A MULTI-STAGE CLUSTER SAMPLING METHOD FOR MONITORING AND EVALUATION OF THE TRACHOMA CONTROL PROGRAMS IN THE TANZANIA

Mathias Kamugisha[1], Upendo J. Mwingira[2], Patric Massae[3], Laura Gower[4], Kesheni Senkoro[5], Andreas Nshala[2], Alphonsina Nanai[6], Haran Mkocha[7], Jeremiah Ngondi[8], George Kabona[2], Mahdi Ramsan[9]

[1]NIMR, Tanga, United Republic of Tanzania, [2]Neglected Tropical Diseases Control Programme/MoHSW, Dar es Salaam, United Republic of Tanzania, [3]KCMC-Trachoma Project, Kilimanjaro, United Republic of Tanzania, [4]Sightsavers, Tanga, United Kingdom, [5]NIMR, Dar es salaam, United Republic of Tanzania, [6]World Health Organization-TZ, Dar Es salaam, United Republic of Tanzania, [7]KTP, Dodoma, United Republic of Tanzania, [8]RTI Tanzania, Dar Es salaam, United Republic of Tanzania, [9]RTI, Dar es Salaam, United Republic of Tanzania

The World Health Organization recommends control of trachoma through surgery, antibiotics, facial cleanliness and environmental improvement (SAFE) Strategy. Baseline survey of trachoma must be undertaken before SAFE implementation and impact surveys are recommended after 3-5 years of SAFE implementation. A multi-stage cluster random sampling design was adopted for surveys of trachoma in 2012. The survey had two components. The first was a baseline survey to estimate prevalence of trachoma and its associated risk factors in 10 districts considered at high risk for trachoma. Twenty (20) villages (clusters) were randomly selectedin each of the baseline survey districts. The second was impact survey of prevalence of trachoma and its associated risk factors in 3 districts following interventions with SAFE from 2005 to2011. In each impact survey district, villages were grouped into three or four sub-districts, configured to comprise at least two Wards thus permitting finer stratification depending on the number of Wards available. Ten villages were selected randomly from each sub-district, giving a total of 30 or 40 villages per district. For both surveys, villages were stratified by Wards with the aim at having equal representation of geographical administrative areas. The total population and households per Ward were used in the process of determining the number of primary sampling unit (village) required for each Ward. At least one village was randomly selected per Ward. Random sampling was used to select the number of villages required for each Ward. In the second stage households required to meet the desired sample were randomly selected in each baseline survey village (36) impact survey village (73). The households were equally allocated to the number of hamlets in each sampled village. In each hamlet, systematic random sampling was used to select the number of household to be surveyed. This survey design has provided the trachoma control programme in Tanzania a reliable method for estimating prevalence of trachoma for programmatic decision making.

1232

INTEGRATED MONITORING AND EVALUATION FOR LYMPHATIC FILARIASIS AND OTHER NEGLECTED TROPICAL DISEASES USING A MULTIPLEX BEAD ASSAY

M. Harley Jenks, Souleymane Sidibe, Brian Chu, Dominique Kyelem, Roland Windtare Bougma, Patrick Lammie

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

Transmission Assessment Surveys (TAS) are conducted to evaluate Mass Drug Administration (MDA) programs for lymphatic filariasis (LF) to determine when the level of incident infection in six to seven year old children is sufficiently low that population drug treatment may be ceased. After MDA is stopped, current WHO recommendations for surveillance are based on repeat TAS, using ICT to detect filarial antigen. We used a multiplex immunoassay that detects antibodies against multiple antigens as an integrated surveillance tool to assay blood spots from 1590 children who participated in a TAS for LF in the Dafra, Karangasso-Vigue, and Lena health districts of Burkina Faso. The multiplex assay is a flexible platform, which provides a facile tool to combine monitoring and evaluation opportunities among multiple NTDs. Using the multiplex antibody assay, LF seroprevalence as measured by detection of Bm14 antibody was 2.7%, compared to antigenemia prevalence by ICT of 0.3%. Although the primary goal of the TAS was to estimate active transmission levels of LF, samples also were tested for antibody against antigens for onchocerciasis, strongyloidiasis and trachoma. In the Burkina Faso evaluation unit, the prevalence of antibodies against Ov16 (onchocerciasis) was 0.3%, the prevalence of antibodies against NIE (strongyloidiasis) was 1.4% and the prevalence of antibodies against trachoma antigens was 2.9% as measured by antibodies against Pgp3 antigen and 3.4% as measured by antibodies against CT694 antigen. The use of the antibody multiplex assay in this context provides a proof of principle of the potential utility of integrated TAS for programmatic monitoring and evaluation for NTDs. With this technology, it is also possible to coordinate NTD monitoring with efforts to evaluate the impact of malaria inventions, vaccine coverage or the health impacts of water and sanitation projects.

1233

UPDATES ON TRACHOMA PREVALENCE IN TANZANIA AS PER 2012 BASELINE SURVEY

Upendo John Mwingira[1], George Kabona[1], Mathias Kamugisha[2], Bernard Kilembe[1], Edward Kirumbi[1], Ibrahim Kabole[3], Damas Deogratias[4], Peter Nyanda[5], Alphonsina Nanai[6], Mwelecele Malecela[2], Maria Chikawe[2], Christina Mbise[7], Mahdi Ramsan[8], Kathryn Crowley[9], Alphonsina Nanai[6], Harran Mkocha[10], Ann Varghese[11], Lisa Rotondo[9], Jeremiah Ngondi[8]

[1]Neglected Tropical Disease Control Program/MoHSW, Dar es Salaam, United Republic of Tanzania, [2]NIMR, Dar es Salaam, United Republic of Tanzania, [3]Sight Savers Tanzania, Dar es Salaam, United Republic of Tanzania, [4]IMA World Health Tanzania, Dar es Salaam, United Republic of Tanzania, [5]Helen Keller International, Dar es Salaam, United Republic of Tanzania, [6]WHO-TZ, Dar es Salaam, United Republic of Tanzania, [7]Sightsavers, Dar es Salaam, United Republic of Tanzania, [8]RTI Tanzania, Dar es Salaam, United Republic of Tanzania, [9]RTI Headquarters, Washington, DC, United States, [10]KTP, Dodoma, United Republic of Tanzania, [11]IMA World Health Headquarters, Washington, DC, United States

Control of blinding trachoma through the surgery, antibiotics, facial cleanliness and environmental improvement (SAFE strategy) is a global initiative that was endorsed by the World Health Organization in 1998. The SAFE strategy utilises surgery for trachomatous trichiasis (TT), antibiotics for mass treatment of active trachoma, and facial hygiene and environmental improvement to reduce transmission of ocular chlamydia. Prior to SAFE implementation, a baseline survey on trachoma prevalence must be undertaken. This survey primarily assists programmes to understand the epidemiology of the disease in order to deliver appropriate treatment and control interventions. As of 2012, only 50 out of 130 districts had been mapped countrywide in a phased approach in 2004 and 2006. In the 2012, another phase of trachoma mapping baseline surveys was done to determine the magnitude of trachoma endemicity and its associated risk factors in the un-surveyed at risk districts of Mbulu, Serengeti, Bariadi, Uyui, Urambo, Karatu, Babati, Arumeru, and Korogwe. A multi-stage cluster random survey sampling was applied whereby 20 villages (clusters) and 36 households per cluster were surveyed. A total of 6,777 households were surveyed and 29,230 participants (93.2% of those enumerated) examined for trachoma signs. Of the 11,417 children aged 1-9 years examined, overall prevalence of trachomatous inflammation-intense (TF) was 1.3% (95% confidence interval [CI] 1.0-1.7). Prevalence of TF varied by district ranging from 0% in Urambo to 2.9% in both Mbulu and Karatu. The proportion of children with a clean face (defined as absence of ocular or nasal discharge) was 84.4%. A total of 17,813 people aged 15 years and above were examined of TT. Overall prevalence of TT was 0.4% (95% CI 0.2-0.6) and varied by district ranging from 0.1% in Bariadi and Urambo to 0.7% in Babati. Based on the survey findings none of the surveyed districts qualify for implementation of the SAFE strategy.

1234

AN ANALYTICAL REVIEW OF ACADEMIC, TECHNICAL AND LABORATORY CAPACITY FOR NEGLECTED TROPICAL DISEASES IN NIGERIA

Patricia N. Okorie[1], Moses J. Bockarie[2], David H. Molyneux[2], Louise A. Kelly-Hope[2]

[1]Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Ibadan, Nigeria, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Nigeria has a significant burden of the neglected tropical diseases (NTDs) that are currently being targeted for control and elimination globally through the preventive chemotherapy strategy. Four main helminth infections, onchocerciasis, lymphatic filariasis (LF), schistosomiasis and soil transmitted helminthiasis (STH) are specifically being targeted with the mass administration of anthelminthic medicines. Nigeria is preparing to scale up its efforts to control and eliminate these NTDs and will require a range of internal technical support and expertise for mapping, monitoring and evaluating, operational research and documenting its success. This study aimed to examine the existing capacity on NTDs in academic and research institutes, and to determine how they may potentially support programmatic activities in Nigeria. Based on published data in the last five years from scientific literature and national institutional reports available in the public domain, all references on studies were systematically compiled into a database recording the institution, department, disease, research type (i.e. molecular, parasitological, immunological, epidemiological and/or entomological), international collaborator institute, journal name and impact factor. All institution locations were mapped, and the filarial disease loiasis also included given its importance to onchocerciasis and LF programmes. A total of 178 publications from 119 journals, highlighted that 47 institutions were involved in NTD research; of these 30 (64%) worked on schistosomiasis, 25 (53%) on onchocerciasis, 25 (53%) on LF, 22 (47%) on STH and 11 (23.4%) on loiasis. The most common type of research was parasitological, and the least was molecular studies. International collaborators were from a few selected institutes in Europe and USA, and publications were generally in low impact journals. This study shows that many institutions are working on NTDs in Nigeria and their capacity could be readily enhanced with training and resources to boost their skills, and increase their range of technical activities and research visibility, which will also help to provide essential technical and laboratory support to the national NTD programmes.

1235

THE ROAD TO TRACHOMA ELIMINATION IS NOT TRAVELED ALONE: SHARED BEST PRACTICES ACROSS MALI, NIGER AND TANZANIA IN DELIVERY OF TRICHIASIS SURGICAL SERVICES FROM 2008-2012

Emily Toubali[1], Seydou Goita[2], Toudja Tchouloum[3], Peter Nyanda[4], Zana Berthé[2], Moses Kabogo[5], Sidi Coulibaly[2], Sanoussi Bamani[6], Boubacar Kadri[7], Upendo Mwingira[8], Chad MacArthur[1]

[1]Helen Keller International, New York, NY, United States, [2]Helen Keller International, Bamako, Mali, [3]Helen Keller International, Niamey, Niger, [4]Helen Keller International, Dar es Salaam, United Republic of Tanzania, [5]Helen Keller International, Mtwara, United Republic of Tanzania, [6]Programme National de Lutte Contre la Cécité, Bamako, Mali, [7]Programme National de Sante Oculaire, Niamey, Niger, [8]National Neglected Tropical Disease Program, Dar es Salaam, United Republic of Tanzania

Mali, Niger, and Tanzania - countries with a history of immensely high trachoma disease burden - have made substantial achievements over the past five years in their trachoma elimination efforts. Although all three countries are in various stages of scaling-up trichiasis surgical services, they have shared similar challenges and lessons learned. A systematic review of monthly program data (2008-2012) reported to Helen Keller International from Mali, Niger, and Tanzania was conducted to identify the fundamental lessons learned and challenges. From these, four themes in best practices were identified: "Leadership and Ownership," "Mobilization," "Quantity with Quality," and "Innovation for Improvement." Leadership and Ownership addressed the necessity of having national-, regional-, and district-level eye heath leaders fully engaged in an intensive leadership role for all aspects of surgical delivery. The direction provided by these individuals and the ownership they assumed served as a catalyst for the highest quality planning, implementation, supervision, and monitoring/evaluation of activities. Mobilization applied to the timely employment of human resources, such as trichiasis surgeons and supervisors; monetary resources to support consumables, equipment, and fuel; trusted community leaders, such as village chiefs and religious figures, to endorse the activities and encourage community participation; and health messaging through community radios, women's groups, and town criers to inform the public about trichiasis camps. Quantity with Quality went hand-in-hand; achieving district-level surgical goals required camps to be strategically planned to maximize output and resources, while simultaneously, quality control measures decreased the probability of post-operative trichiasis. Innovation for Improvement allowed existing strategies to be improved and perfected, with the development of new strategies to overcome evolving challenges. Implemented concurrently, these best practices will continue to guide each country in their efforts to eliminate blinding trachoma.

1236

ASSESSING DATA QUALITY OF NEGLECTED TROPICAL DISEASES PROGRAMS IN UGANDA

Edridah Muheki[1], Charles Wamboga[1], Ruth Magola[1], Timothy Wakabi[2], Harriet L. Namwanje[2], Kathryn L. Zoerhoff[3], Pamela S. Mbabazi[4]

[1]Vector Control Division, Uganda Ministry of Health, Kampala, Uganda, [2]RTI International, Kampala, Uganda, [3]RTI International, Nairobi, Kenya, [4]World Health Organization, Geneva, Switzerland

High coverage is essential for the success of preventive chemotherapy (PC) programs targeting NTDs, and drug distribution data is routinely reported. However, national NTD programs have only rarely conducted formal data quality assessments (DQA) to evaluate the quality of reported data and data management systems, despite the widespread use of DQA for other public health interventions. The Uganda National NTD Program, collaborating with RTI-ENVISION and WHO, conducted its first DQA for NTDs at 16 service delivery points (SDP) in 4 sub-counties of 4 districts treated through PC in 2012. This DQA consisted of verifying available reported results through comparison with recounted values for 5 indicators at village, sub-county, and district; as well as interviewing individuals involved in PC data compilation and reporting to qualitatively assess the PC data management system. We discovered that reports were available 75% of the time, and 87% of reported results were verified at the district level. The interviews with community medicine distributors and their supervisors revealed somewhat limited understanding how to complete reporting forms at SDP and sub-county levels, which sometimes resulted in inaccurate reported results at these lower levels (range of average DQA data verification factor: 0.6-2). Standard tools for reporting were identified and fairly consistently used at the various levels; we found that in most cases, guidance was provided on how to complete tools, indicators to be reported, and to whom reports should be submitted, although many respondents were not informed when reports should be submitted. These findings suggest that the quality of the monitoring system is satisfactory, although additional training and supportive supervision for data compilation is needed to ensure accuracy of reported results. In the future, the national NTD program in Uganda, with support from partners, should strengthen data management training and supportive supervision; establish clear timelines for reporting; and develop job aides to facilitate data management and use.

1237

COORDINATED ASSESSMENT OF SOIL-TRANSMITTED HELMINTHIASIS AND LYMPHATIC FILARIASIS THROUGH TRANSMISSION ASSESSMENT SURVEYS IN BENIN AND TONGA

Brian K. Chu[1], David G. Addiss[1], Wilfrid Batcho[2], Ameyo M. Dorkenoo[3], Malakai Ake[4], Eva Maffi[4], Katherine Gass[1]

[1]The Task Force for Global Health, Decatur, GA, United States, [2]Ministère de la Santé, Cotonou, Benin, [3]Ministère de la Santé, Lome, Togo, [4]Ministry of Health, Nuku'alofa, Tonga

Mass drug administration (MDA) for lymphatic filariasis (LF) programs has delivered over 2 billion treatments of albendazole plus either ivermectin or DEC, including many to communities co-endemic for soil-transmitted helminthiasis (STH), resulting in considerable reduction of the prevalence of both diseases. A transmission assessment survey (TAS) using lot quality assurance sampling, designed to determine if MDA for LF can be stopped in an evaluation unit (EU) after five rounds of annual treatment, may provide a valuable opportunity to integrate assessment activities by evaluating the impact of MDA on STH through a coordinated survey approach. Pilot studies conducted in Benin and Tonga assessed the feasibility of combining an STH survey alongside a TAS. Of the 30 schools (clusters) selected for a TAS in each EU, a subset of 5 schools per STH ecological zone was randomly selected, according to World Health Organization (WHO) guidelines, for the coordinated survey. In Benin, a total of 519 children were sampled in 5 schools and 22 (4.2%) were found positive for STH infection (*Ascaris lumbricoides, Trichura trichiura,* or hookworm) measured by the Kato-Katz test. All positive cases were classified as light intensity under WHO criteria. STH infection in children 6-7 years old (recommended by TAS) was not significantly different from those 8- 9 years old (recommended for STH by WHO guidelines). In Tonga, 10 of 30 schools were chosen for the coordinated TAS and STH survey resulting in 114 of 552 (20.7%) positive cases. All children sampled were aged 6-7 and all infections were of light intensity with the exception of one moderate *T. trichiura* case. Synchronous assessment of STH with TAS provides a convenient, well-timed assessment of infection prevalence to determine ongoing treatment decisions at the time MDA for LF may be stopped. The field experiences in both Benin and Tonga also highlighted potential time and cost savings through a coordinated approach. Refinement of a coordinated TAS and STH sampling strategy should be pursued in addition to further testing of alternate diagnostics to Kato-Katz for improved survey logistics.

1238

AFRICAN PROGRAM FOR ONCHOCERCIASIS CONTROL 1995-2010: IMPACT OF ANNUAL IVERMECTIN MASS TREATMENT ON OFF-TARGET NEGLECTED TROPICAL DISEASES

Stanimira P. Krotneva[1], **Luc E. Coffeng**[1], Mounkaila Noma[2], Honorat G. Zouré[2], M. Bakone[2], Uche V. Amazigo[2], Sake J. de Vlas[1], Wilma A. Stolk[1]

[1]Department of Public Health, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands, [2]African Programme for Onchocerciasis Control, Ouagadougou, Burkina Faso

Since its initiation in 1995, the African Program for Onchocerciasis Control (APOC) has had a substantial impact on the prevalence and burden of onchocerciasis through annual ivermectin mass treatment. Ivermectin is a broad spectrum anti-parasitic agent which also has an impact on other co-endemic parasitic infections. In this study, we assessed the additional impact of APOC activities on the burden of such off-target diseases. We first reviewed the literature to identify off-target infections that are likely to be impacted based on their endemicity in APOC countries and ivermectin efficacy against such infections, if any. Based on this review, we selected soil-transmitted helminthiases (STH; ascariasis, trichuriasis, hookworm, and strongyloides), lymphatic filariasis (LF), and epidermal parasitic skin diseases (EPSDs) as the most important, potentially impacted off-target infections. Using data on the number of given treatments and the latest estimates of the burden of disease, we calculated the impact of APOC activities on off-target infections in terms of disability adjusted life years (DALYs) averted. We estimated that between 1995 and 2010, annual ivermectin mass treatment has cumulatively averted about 500 thousand DALYs from co-endemic STH infections, LF, and EPSDs. This impact comprised an additional 5.5% relative to the total burden averted from onchocerciasis (9 million DALYs), and suggests that the overall cost-effectiveness of APOC is even higher than previously estimated.

1239

HEALTH-EDUCATION PACKAGE TO PREVENT WORM INFECTIONS IN CHINESE SCHOOLCHILDREN

Franziska A. Bieri[1], Darren J. Gray[1], Gail M. Williams[1], Giovanna Raso[2], Yuesheng Li[3], Yuan Liping[4], He Yongkang[4], Robert S. Li[1], Fengying Guo[4], Shengming Li[4], Donald P. McManus[3]

[1]University of Queensland, Brisbane, Australia, [2]Swiss Tropical and Public Health Institute, Basel, Switzerland, [3]Queensland Institute of Medical Research, Brisbane, Australia, [4]Hunan Institute of Parasitic Diseases, Yueyang, China

A third of the global population, mainly in developing countries, is infected with soil-transmitted helminths (STHs). Infection with these intestinal parasitic worms is associated with poverty in rural locations, inadequate sanitation and waste disposal, a lack of clean water, and poor hygiene and is common in areas with limited access to health care and preventive measures. Major success in preventing STH infections in Chinese schoolchildren as the result of a health education package we implemented incorporating a cartoon video. The study, undertaken in Hunan Province, showed a 50% efficacy in preventing the incidence of STH infection after a cluster randomized controlled trial in 38 schools involving 1718 schoolchildren, as previously published. The intervention proved highly successful with a significant impact evident across all outcome measures. We are now evaluating the efficacy of the educational package in two additional cluster-randomized controlled trials in China and in the Philippines. In China, we evaluate the package in a high STH prevalence setting in Yunnan Province. For the Philippines, the educational package will be culturally adapted so as to assess its efficacy in another Southeast Asian setting. The overall research objectives, study design and the main results of the Hunan Province trial will be described briefly and selected scenes of the animated narrative cartoon video, which forms the basis of the education package, will be shown. New STH control strategies are urgently needed, since current control efforts focusing on mass drug administration have been shown to be unsustainable due to rapid reinfection. The video-based educational package we have developed and trialled successfully provides a promising new tool for integrated STH control.

1240

THE MODELLING OF SCHISTOSOMIASIS ELIMINATION

Michael D. French[1], Joanne Webster[1], Wendy Harrison[1], Deirdre Hollingsworth[2], James Truscott[3]

[1]Schistosomiasis Control Initiative, London, United Kingdom, [2]University of Warwick, Warwick, United Kingdom, [3]Imperial College London, London, United Kingdom

In the last few years momentum has shifted from a target of controlling schistosomiasis morbidity to aiming for possible elimination. However, there is currently an insufficient understanding of schistosomiasis transmission dynamics and the combination of interventions that may be required to reach elimination (such as mass drug administration (MDA), water, sanitation, and hygiene improvements (WASH), and snail control). This talk will describe the work that that the Schistosomiasis Control Initiative (SCI) and its London Centre for Neglected Tropical Disease Research (LCNTDR) partners is undertaking on this area, as part of a new operational research grant. A mathematical model of transmission dynamics for helminths is being developed in a related project within the LCNTDR, headed by Prof. Sir Roy Anderson. This is being constructed specifically for soil-transmitted helminths but the same underlying model will be utilised and extended to explore schistosomiasis transmission. Currently available datasets (those of the SCI others) will be used to estimate schistosomiasis-specific transmission and infection parameters for the model. Parameters will also be estimated using incoming data from trials on MDA, WASH, and snail control, as well as using figures taken from the published literature. It is hoped that the outputs of the modelling objective will help identify: the transmission breakpoint of infection in a population; the contribution of young children to transmission and the optimum approach for them; and the suggested schistosomiasis elimination strategies for different geographies and populations. It is also hoped that the outputs will be used to directly inform guidelines for the helminth control programmes.

1241

METALLOPROTEINASES 2 AND 9 ARE DIFFERENTIALLY EXPRESSED IN PATIENTS WITH INDETERMINATE AND CARDIAC CLINICAL FORMS OF CHAGAS DISEASE

Juliana de Assis Estanislau[1], Rafaelle Christine Fares[2], Luciana Ribeiro Garzoni[3], Mariana Caldas Waghabi[3], Roberto Magalhães Saraiva[3], Nayara Ingrid Medeiros[1], Roberta Oliveira Prado[2], Fernanda Fortes de Araújo[2], Andréa Teixeira-Carvalho[4], Giovane Rodrigo Sousa[1], Manoel Otávio Costa Rocha[1], Rodrigo Correa Oliveira[4]

[1]Federal University of Minas Gerais, Belo Horizonte, Brazil, [2]Centro de Pesquisas Rene Rachou, Belo Horizonte, Brazil, [3]FIOCRUZ, Rio de Janeiro, Brazil, [4]Centro de Pesquisas René Rachou, Belo Horizonte, Brazil

Dilated chronic cardiomyopathy (DCC) from Chagas disease is associated with myocardial remodeling and interstitial fibrosis, resulting in extracellular matrix (ECM) changes. In this study, we characterized for the first time, the serum MMPs 2 and 9 levels, their enzymatic activities as well as their main cell sources in peripheral blood from patients presenting the indeterminate (IND) or cardiac (CARD) clinical forms of Chagas disease. Our results showed that serum levels and enzymatic activity of MMP-9 are associated with the severity of Chagas disease. The analysis of MMPs production by T lymphocytes showed that $CD8^+$ T cells are the main source of both MMP-2 and MMP-9 molecules. Using a new 3-dimensional model of fibrosis we observed that serum from patients with Chagas disease induced an increase in the extracellular matrix components in cardiac spheroids. Furthermore, MMP-2 and MMP-9 showed different correlation

with matrix proteins and inflammatory cytokines in patients with Chagas disease. Our results suggest that MMP-2 and MMP-9 show distinct activities in Chagas disease pathogenesis. While MMP-9 seems to be involved with the inflammation and cardiac remodeling of Chagas disease, MMP-2 does not correlate with inflammatory molecules.

1242

CHARACTERIZATION OF INFECTIVITY AND IMMUNOGENESIS OF GENETICALLY ALTERED LIVE-ATTENUATED *LEISHMANIA DONOVANI* PARASITES IN THE MOUSE LIVER: ROLE OF KUPFFER CELLS

Dwann Davenport, Ranadhir Dey, Jacqueline Araujo Fiuza, Hira Nakhasi, Robert Duncan

Food and Drug Administration, Bethesda, MD, United States

Leishmania donovani, a kinetoplastid parasite, causes visceral leishmaniasis, a fatal disease if left untreated. *L. donovani* has a digenic lifestyle and alternates from the extracellular promastigotes form in the gut of the sandfly vector to the intracellular amastigote form in macrophages of the vertebrate host where it resides in internal organs, such as the liver, spleen, and bone marrow. Kupffer cells (KCs), macrophages of the liver, are targets of *L. donovani* and play a major role in its clearance. However, the role of the liver in immunoprotection is not completely understood. As potential vaccine candidates, our lab developed attenuated *L. donovani* parasites through gene knock-outs: centrin 1 mutant (LdCen-/-) and p27 mutant (Ldp27-/-). The aim of this study is to evaluate the infectivity and immune response to live-attenuated parasites in KCs *in vitro*. Cells were harvested from the livers of BALB/c mice after perfusion to remove circulating red blood cells and leukocytes. The livers were disrupted to form single cell suspensions, and fractionated through density centrifugation to isolate intrahepatic leukocytes (IHLs). The IHLs were cultured, and KCs were isolated from the IHL population by their adherence to plastic, which averaged ~90% of the IHL population. KCs were characterized by flow cytometry using cell surface markers CD45 and F4/80. These cells were used for *in vitro* infection with *L. donovani* wild-type, LdCen1-/-, or Ldp27-/- parasites. The data on infection as indicated by the parasite number and immune response by measuring NO and secreted cytokines will be presented. Further evaluation of the pathogenesis and immune response to *L. donovani* wild-type, LdCen1-/-, or Ldp27-/- parasites in the liver will be studied by *in vivo* infection of mice.

1243

ACTIVATION OF IRE1-MEDIATED SIGNALING PATHWAY IN CACO-2 CELLS INFECTED WITH *TRYPANOSOMA CRUZI* OR TREATED WITH LPS

Kumud Dahal[1], Raj Wadgoankar[2], Haseeb M.A.[2]

[1]State University of New York Downstate Medical Center, Brooklyn, NY, United States, [2]State University of New York Downstate Medical Center, Brooklyn, NY, United States

Lipopolysaccharide (LPS) induced TLR-4 activation leading to kinase inositol-requiring enzyme 1 (IRE1)-mediated signaling pathway has been shown to upregulate inflammatory response targets while leaving the canonical unfolded protein response (UPR) targets uninduced. Activation of endoplasmic reticulum (ER) stress sensor IRE1a and its downstream target, the transcription factor, X-box binding protein 1(XBP1) acts synergistically with TLR-4 for optimal and sustained production of proinflammatory cytokines in macrophages**.** UPR is a homeostatic signaling response that attempts to minimize ER-mediated protein folding stress by globally diminishing translation, and increasing the folding and secretory capacity of ER. UPR is typically occurs in metazoan eukaryotes, but a distinct form is observed in kinetoplastids. Conventional UPR proteins, including IRE1a, have not been detected in kinetoplastids by standard ortholog detection tools. We sought to explore the role of *Trypanosome cruzi* and/or LPS in activation of IRE1b, one of the UPR proteins, that is typically expressed in colonic epithelial cells. We prepared extracts of untreated Caco-2 cells, and Caco-2 cells infected with *T. cruzi* (Brazil strain; 1 x 10^7/mL), treated with LPS (100 ng/ml) or cells first infected with *T. cruzi* and then treated with LPS. In each instance, *T. cruzi* was allowed to develop for five days and LPS treatment lasted 12 hours. The cell extracts were subjected to polyacrylamide gel electrophoresis and transferred to polyvinylidane difluoride membrane. The blots were treated with anti-IRE1b which was detected by a chemiluminescence assay. We detected IRE1b protein in extracts of Caco-2 cells after each or both treatments. Our findings suggest that both *T. cruzi* and LPS independently induce IRE1b mediated-ER stress response in Caco-2 cells. This may be a novel innate immune signaling pathway, independent of the classical UPR response, that warrants further investigation.

1244

GENETICALLY MODIFIED LIVE ATTENUATED *LEISHMANIA DONOVANI* PARASITES INDUCE PROTECTIVE IMMUNE RESPONSE *IN VITRO* IN BONE MARROW DERIVED MACROPHAGES FROM YOUNG AND AGED MICE

Parna Bhattacharya[1], Ranadhir Dey[1], Michael Kruhlak[2], Sreenivas Gannavaram[1], Hira L. Nakhasi[1]

[1]Food and Drug Administration, Bethesda, MD, United States, [2]National Institute of Cancer, National Institutes of Health, Bethesda, MD, United States

Leishmaniasis causes significant morbidity and mortality worldwide and age of the infected individuals appears to be critical in determining the clinical outcome of the infection. Previously we showed that genetically modified live attenuated *Leishmania donovani* parasite cell lines (LdCen$^{-/-}$ and Ldp27$^{-/-}$) induce a strong protective cellular immune response against wild type *Leishmania* infection in mice. In this study we explored whether there is age related difference in immune response to the two live attenuated parasites in bone marrow derived macrophages (BMDM) from young (2 months) and old (18 months) mice. We observed that both these cells lines induce Nitric Oxide (NO) and concomitant suppression of arginase activity, in BMDMs derived from both age groups compared to wild type parasite. The enhanced NO production correlated with the increased phosphorylation of p38MAPK and down regulation of phosphorylation of ERK, components involved in regulating pro-inflammatory cytokine response. Next we evaluated the effect of live attenuated parasite infection on the membrane architecture of the BMDMs from young and aged mice. There is neither quenching of cholesterol from the membranes nor change in membrane fluidity as is observed with wild type infection of such macrophages. In addition there is augmentation of the antigen presentation capability of young and aged mice derived BMDMs by prominently restoring the MHC-II architecture. Overall the study suggests that live attenuated parasites generate an immune response in BMDM isolated from young and aged groups of mice similar to that has been observed in protection against *Leishmania* infections. Therefore these studies suggest that live attenuated parasites can be efficacious irrespective of the age. Future studies will focus on evaluating the efficacy of these attenuated parasites *in vivo* in both young and aged mice against *Leishmania* infection.

1245

IDENTIFICATION OF NEW ANTIGENS FROM *TRYPANOSOMA CRUZI* GENOME BY IMMUNOINFORMATICS ANALYSIS AND THE EVALUATION OF THE RECALL RESPONSE OF SPLENOCYTES FROM INFECTED MICE

Christian Teh-Poot, Evelyn Tzec-Arjona, Maria Jesus Ramirez-Sierra, Miguel Rosado-Vallado, **Eric Dumonteil**

Universidad Autonoma de Yucatan, Merida, Yucatan, Mexico

Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi*, and activation of CD8+ T cell is crucial for a protective immune response. Therefore, the identification of antigens with epitopes restricted to MHC class I is necessary for vaccine development against *Trypanosoma cruzi*. In

this study, immunoinformatic programs were used to predict epitopes for H-2Dd and H-2Kd. In a first step of the analysis, we identified 172 epitopes with MHC binding potential using the programs NetMHC and RANKPEP. The proteins sequence containing these epitopes were re-analyzed with other programs (SYFPEITHI BIMAS-HLA-I ProPred, MAPPP, ANNPred, compred, SVMHC, IEBD and Preded) to generate a combined consensus of prediction. We selected 26 epitopes that were predicted by the highest number of programs and the best consensus of prediction for validation. We measured the IFNγ recall response of splenocytes from *T. cruzi* infected mice by ELISA following stimulation with the selected peptides. The results show that 10/26 epitopes induce IFNγ production, indicating that these epitopes can cause T cell activation. This suggests that these epitopes are processed and presented by MHC class I by antigen-presenting cells during natural infection by *Trypanosoma cruzi*. 4/10 epitopes confirmed to correspond to proteins with enzymatic functions, and 6/10 correspond to proteins with unknown functions. These epitopes are new potential vaccine candidates against Chagas disease.

1246

DOES CLUSTERING EXIST IN THE TCI DTU OF *TRYPANOSOMA CRUZI,* A REVISION OF THE MINI-EXON GENE (SL-IR)

Claudia P. Herrera[1], Christian Barnabé[2], Simone F. Brenière[2]

[1]*Tulane University, New Orleans, LA, United States,* [2]*Institut de Recherche pour le Développement (IRD), Montpellier, France*

Trypanosoma cruzi includes six discrete typing units (DTUs) TcI-TcVI and a seventh one named Tcbat. Previous studies based on intergenic sequences of the mini-exon gene (SL-IR) have identified within TcI, five genotype groups presenting specific epidemiological properties. Given the epidemiological importance of TcI, a DTU that has a very wide geographical distribution throughout the endemic area for Chagas disease, we conducted an exhaustive revision of the sequence variability of the SL-IR, using 244 TcI partial sequences from isolates, cellular or molecular clones, from 11 Latin American countries. First, the evolutionary branching between strains was examined by analyzing only the single nucleotide polymorphism (SNP) deleting the microsatellite region and the gaps. After haplotype reconstruction using the PHASE algorithm, because of the presence of several ambiguous nucleotides in the SNP region, a total of 131 different haplotypes were obtained for network construction using median-joining (MJ) method. The topology reveals how difficult it is to identify an obvious structure in TcI for most of the parameters examined; somewhat genetic and geographical structures exist, but no structure was depicted with cycle and host origins. Then the variability of the microsatellite region (MS), previously used to identify the five TcI genotype groups, was reanalyzed using principal component analysis (PCA); similarly, the two-dimensional projection of the 26 depicted genotypes, does not make it possible to infer clear clustering. Indeed, the long-lasting evolution with possible recombination events, the occurrence of several waves of geographical dispersions (old and recent), and the high flow of strains between sylvatic and domestic cycles partially hide the major evolutionary trends within TcI. Moreover, we identified several problems in previous analyses, and concluded that in absence of supplementary studies of TcI phylogeny with other genetic markers, it is hazardous to use only the mini-exon intergenic region as a relevant marker of the substructure within TcI.

1247

LEISHMANIA DONOVANI UFM1-/- UFSP-/- DOUBLE NULL MUTANTS AS POTENTIAL LIVE ATTENUATED VACCINES

Sreenivas Gannavaram, Jacqueline Fiuza, Hira L. Nakhasi

Food and Drug Administration, Bethesda, MD, United States

Leishmaniasis is a spectrum of diseases caused by protozoan parasites belonging to several different *Leishmania* species. There are no effective vaccines against leishmaniases. Recent estimates have shown that an anti-leishmanial vaccine with even a 50% efficacy and protection spanning a 5 year period would still be cost-effective compared to currently available chemotherapies. Previous vaccination approaches including killed *Leishmania* parasites, subunit vaccines or DNA vaccination have not yielded long lasting immunity. We have evaluated genetically attenuated *L. donovani* parasites as vaccine candidates. Such genetically attenuated parasites have been shown to confer robust protection against subsequent *Leishmania* infection in animal models. However, the live-attenuated vaccine approach faces formidable obstacles of demonstration of safety in clinical use. To address the safety of live attenuated parasites, we have developed a *L. donovani* cell line lacking two genes Ufm1 and Ufsp involved in *Leishmania* ubiquitination pathway. Ufm1 (ubiquitin-fold modifier 1) is a ubiquitin like protein that conjugates to parasite proteins in *L. donovani*. This conjugation is initiated by the Ufm1 processing activity of the enzyme Ufsp. Gene deletion experiments have shown that lack of either Ufm1 or Ufsp affect the growth of amastigote stages. The double gene knockout mutants similarly have shown severe growth reduction in the amastigote stage. Infection experiments in Balb/C mice showed a 6-log fold difference in the parasite burden (LdUfm1-/-Ufsp-/- versus wild type) at 8 week post infection in the spleens. These results demonstrate that deletion of Ufm1 and Ufsp genes leads to a strong attenuation of virulence. Further, the use of double gene knockout mutants have the advantage of limiting the probability of recombination and thus reversion to virulent phenotype since the two genes are located on separate chromosomal *loci* (Ufsp on 34 and Ufm1 on 16). Results on the characterization of immunopathology of the double knockout mutants will be discussed.

1248

MOLECULAR EPIDEMIOLOGY OF CHAGAS DISEASE IN SOUTHEASTERN LOUISIANA: ANALYSIS OF SAMPLES FROM PERIDOMESTIC RODENTS AND KISSING BUGS

Claudia P. Herrera, Catherine S. Nation, Samuel B. Jameson, Pierre Buekens, Dawn M. Wesson

Tulane University, New Orleans, LA, United States

The protozoan parasite *Trypanosoma cruzi*, causative agent of Chagas disease, infects at least 8 million people in Latin America and is estimated to cause ~13,000 deaths per year. *T. cruzi* is endemic throughout Latin America and in the southern half of the United States, but Chagas disease occurs primarily in areas where human populations have contact with domestic and peridomestic triatomine vector species associated with infected vertebrate reservoirs. Some autochthonous cases have also been reported in the southern United States. Although bugs are directly involved in the process of active transmission of the pathogen, it is important to consider all the risk factors associated with the vector-host-parasite relationship. Tissues from thirty rodents captured in a rural area of New Orleans, LA were analyzed. The samples, including heart, liver, spleen and muscle, were examined for the presence of *T. cruzi*. We also examined five recently collected triatomines (*Triatoma sanguisuga*). Analysis of > 500 additional triatomine specimens is ongoing and will be reported at the meeting. In the rodents, 100 samples corresponding to different tissues were taken for DNA extraction using QIAmp® DNA extraction kit (Quiagen). The molecular characterization of the tissues obtained was performed by amplifying the kDNA minicircle and the mini-exon gene's intergenic region-SL-IR. Preliminary data showed that 18/30 (60%) rodents were positive for *T. cruzi* and 3/5 (60%) of the triatomines also tested positive. At least three distinct strains of *T. cruzi* were present in the samples. The amplification from *T. cruzi* DNA extracted from tissues showed an interesting mix of infections among TcI and non-TcI, possibly exhibiting a tissue-specific strain association. These results show the importance of determining *T. cruzi* strain associations with different triatomine and vertebrate host species so as to better understand the eco-epidemiology of *T. cruzi* in Latin America and in the southern Unites States. Ultimately, this will bring about improved projections of risk of infection for humans in differing environments.

1249

CONSIDERABLE PROPORTION OF *LEISHMANIA BRAZILIENSIS* RRNA MOLECULES ARE POLYADENYLATED

Marlene Jara Portocarreo, Jorge Arevalo

Instituto de Medicina Tropical Alexander von Humboldt, Lima, Peru

Leishmania parasites are ancestral eukaryotes with unusual characteristics like polycistronic transcription and RNA trans-splicing. Like other eukaryotes, their RNA ribosomal genes are tandemly repeated and transcribed by RNA polymerase I. Unlike other eukaryotes, *Leishmania* ribosomes have rRNA molecules of 18S, 5.8S, and 28S, with the latter one being split into six rRNAs (α.γ, β, δ, ζ and ε). The polyadenylation is a post-transcriptional process well known for mRNA but scarcely reported for rRNA. Our previous work on *L. braziliensis* and *L. donovani* demonstrated that at least the rRNA 28S ε undergo the polyadenylation process and that its relative abundance varies in *Leishmania* promastigote and amastigote stages. To determine if all rRNA gene subunits are subjected to polyadenylation, we evaluated the 18S rRNA, 5.8S rRNA and all the subunits homolog to 28S rRNA at stationary and logarithmic phase promastigotes of the *L. braziliensis* strain MHOM/BR/75/M2904. We found that all the rRNA subunits were polyadenylated. Moreover, we quantified the absolute amount of polyadenylated and non-polyadenylated rRNA of the sub-units 18S, 5.8S and 28S α by Reverse Transcription-Real time quantitative PCR. In the logarithmic promastigotes, the percentage of polyadenylated rRNA 18S, rRNA 5.8S and rRNA 28S α were 0.378 ± 0.02 (mean ± standard deviation), 4.55 ± 0.43 and 13.86 ± 0.95, respectively. The stationary promastigotes had higher percentages of polyadenylated rRNA 18S (0.704 ±0.29, *P*=0.064) and rRNA 5.8S (5.69 ± 0.28, *P*=0.045) than the logarithmic promastigotes, whereas the 28S α did not show any significant differences between log and stationary promastigotes. These findings confirm a remarkable fact of *Leishmania* rRNA gene expression (also present in *L.amazonensis*, data not shown) and it is related to the parasite growth. The biological role of this phenomenon remains unknown but its wide conservation in the genus *Leishmania* indicates it is an important one.

1250

PHENOTYPIC CHARACTERISTICS OF ACUTE CHAGASIC MYOCARDITIS AMONG C57 AND BALB/C MICE

Andrés F. Henao-Martínez, Anne H. Agler, Timothy A. McKinsey, David A. Schwartz, Ivana V. Yang

University of Colorado Denver, Aurora, CO, United States

Chagasic disease is a notable neglected tropical disease with high morbidity in Latin America and among immigrants to the US. The primary mechanism of mortality is cardiomyopathy and sudden death. Acute chagasic myocarditis is consistently found in acute infections but little is known about its contribution to chronic forms of cardiomyopathy and what host factors play a role in acute myocarditis. The aim of this study was to phenotypically characterize two strains of mice with differential susceptibility to acute chagasic infection and correlate strain phenotypes with heart tissue gene expression. Laboratory mouse Tulahuen strain of *Trypanosoma cruzi* was grown in 3T3 fibroblast cell culture and tissue-derived trypomastigotes (TCT) were harvested from supernatant. C57 and Balb/c mice were injected intraperiotneally with 0 or 150-200 TCT. Weekly, mice were weighed and *parasitemia* was monitored via retro-orbital blood sample. At 4 weeks Brain natriuretic peptide (BNP) and Troponin were measured in plasma and echocardiograms were obtained. 4-week mortality was 56.3% and 12.5% for Balb/c and C57 (p=0.009), respectively. Infected Balb/c mice lost more weight than infected C57 mice (p=0.018). Parasitemia peaked at 2 weeks, but was not significantly different between strains due to high variation in counts: 500,781 ± 866,464 (Balb/c) vs. 140,625 ± 280,606 (C57) parasites/ml (p=0.12). For infected mice, BNP and troponin levels were not significantly different between strains, but BNP differed from uninfected mice. Echocardiograms demonstrated differences in heart rate in BALB/c vs. C57 mice: 413 vs. 476 bpm, (p=0.0001) and stroke volume: 31.9 ± 9.3 vs. 39.2 ± 5.5 µl (p=0.03); therefore in cardiac output: 13.1 ± 3.5 vs. 18.7 ± 3.2 µl/min (p=0.002). There are relevant susceptibility and hemodynamic differences between these strains of mice during acute chagasic infection. Further characterizations of heart tissue histopathology, immunohistochemistry and gene expression will investigate possible host factor determinants for acute chagasic myocarditis.

1250A

QUANTITATIVE KDNA ASSESSMENT DURING TREATMENT OF MUCOSAL LEISHMANIASIS AS A POTENTIAL BIOMARKER OF OUTCOME

Marlene Jara[1], Braulio M. Valencia[1], Milena Alba[1], Vanessa Adaui[1], Jorge Arevalo[1], Alejandro Llanos-Cuentas[1], **Andrea K. Boggild**[2]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]University of Toronto, Toronto, ON, Canada

Mucosal leishmaniasis (ML) is a disfiguring manifestation of infection with *Leishmania* (Viannia) *spp.* As there is no known biomarker of treatment outcome in ML, we evaluated the concentration of kinetoplast minicircle DNA (kDNA) by cytology brush quantitative PCR before, during, and after treatment of ML in Peruvian patients. ML lesions were sampled by cytology brushes for quantitative PCR at enrolment, days 14 and 21_28 of therapy, and 3-, 6-, or 12-mos after treatment. Parasite concentration in tissue was correlated to demographic, clinical, and parasitologic factors. Twenty patients completed follow-up: 12 men and 8 women, with median age of 37 yrs (range 18_78 yrs). Fifteen patients were treated with sodium stibogluconate, and 5 with amphotericin B. Cure was achieved in 17 patients, while 2 patients failed multiple courses of therapy. Clinical outcome is unknown in 1 patient. Mean parasite load (PL) at enrolment was 85,614.8 ± 60,427.3 parasites per ug of tissue DNA (par/ug tDNA). Three patterns of quantifiable kDNA during therapy and follow-up emerged: pattern 1 (N=10) was characterized by a mean PL of 170,867 ± 117,482.6 at enrolment, with sequential decline in PL during and after therapy until kDNA was undetectable. Pattern 2 (N=4) was characterized by mean PL of 566.4 ± 306.4 at enrolment, with clearance of detectable kDNA by D14 of treatment, followed by an increased PL by D21-28 of treatment to 80.4 ± 32.1 par/ug tDNA. Pattern 3 (N=6) was characterized by mean PL of 226.7 ± 116.1 at enrolment, with clearance of detectable kDNA during treatment, followed by increased PL by 6-mos follow-up to 36.6 ± 13.1 par/ug tDNA. Both patients who failed treatment demonstrated Pattern 1. Patterns 2 and 3 were associated with granulomatous inflammation (p=0.02). Younger age (33.5 vs. 64 yrs, p=0.10) and shorter ML duration (20.5 vs. 48 mos, p=0.11) are potentially correlated to sequential clearance (pattern 1). Baseline PL, sex, exposure duration, lesion number, and ML location were not correlated to pattern of PL. We have demonstrated that the concentration of parasite kDNA in ML can be quantified by cytology brush sampling and quantitative PCR during and after treatment. Interim analysis demonstrates 3 distinct patterns of PL during and after treatment, which warrant further investigation. Granulomatous inflammation may predict rebound of PL during or after treatment, though the clinical significance of this rebound is presently unknown.

1251

COMPARISON OF TWO COMBINATION PARASITE LACTATE DEHYDROGENASE-BASED RAPID TESTS FOR THE DIAGNOSIS OF MALARIA DUE TO *PLASMODIUM KNOWLESI* AND OTHER *PLASMODIUM* SPECIES IN SABAH, MALAYSIA

Matthew J. Grigg[1], T. William[1], B. E. Barber[1], U. Parameswaran[1], T. W. Yeo[2], N. M. Anstey[2]

[1]Queen Elizabeth Hospital, Kota Kinabalu, Sabah, Malaysia, [2]Menzies School of Health Research and Charles Darwin University, Darwin, Australia

Plasmodium knowlesi human infection has been reported throughout South-East Asia, and is the most common cause of severe malaria in parts