sources. However, there is growing evidence suggesting the adaptation of Anopheline species to polluted breeding habitats in urban settings. In Ghana, *An. gambiae* has been found breeding in much polluted water bodies leading to an increase in urban malaria cases. This adaptation may pose challenges to the already underfunded malaria control programs. Thus, this study aims at understanding the molecular and genetic basis of this adaptation and evaluating the differences and expression of genes involved in insecticide detoxification in *An. gambiae* s.s. Three Cytochrome P450 genes (CYP_6P3, CYP_4H19 and CYP_4H24), one Glutathione S-transferase gene (GSTD_1-4) and one ABC Transporter gene (ABCC_11), were analysed to determine their expression levels in the larval and adult populations in 5 selected breeding sites, in urban Accra, Ghana. The results revealed that generally the fold expression of these genes was higher and significant in the larvae compared to the adults. The fold expressions, however, varied between sites. With the exception of GSTD 1-4, the expression of the other genes was significantly higher in the most polluted site compared to the other sites. Also, there was significant correlation between ABCC_11, GSTD 1-4, CYP_4H24 and most water quality parameters of the study sites. Analysis of enzyme activity of α-esterase, monooxygenases, glutathione S-transferase and acetylcholinesterase revealed higher and significantly different enzyme activity in larval and adult populations. These results suggest that detoxification enzymes could be involved in adaptation to polluted breeding sites. While the increased enzyme activities observed could be due to functional plasticity, it has been hypothesized that such an adaptive plasticity might continuously evolve to maximize the adaptation of mosquito larvae to breeding sites that are chemically changing. The results may also suggest that perhaps some other mechanisms are involved, which require further studies.

1249

IMPROVING SOCIOECONOMIC EQUITY IN INSECTICIDE-TREATED BEDNETS (ITNS) ACCESS, OWNERSHIP AND USE IN RWANDA FROM 2000-2010

Lia S. Florey[1], Cameron Taylor[1], Rebecca Winter[1], Ana Claudia Franca-Koh[1], Ana Claudia Franca-Koh[2], Samantha Herrera[2], Jui Shah[2], Yazoume Ye[2]

[1]ICF International - The DHS Program, Rockville, MD, United States, [2]ICF International - MEASURE Evaluation, Rockville, MD, United States

Enabled by significant increases in funding for malaria control, remarkable scale-up of malaria control interventions has occurred over the past decade in sub-Saharan Africa. Despite high national coverage with interventions such as insecticide-treated bednets (ITNs), distribution is not always equitable across the population, with coverage varying by households' socioeconomic status. In Rwanda, ownership of ITNs increased substantially from 15% to 82% between 2005 and 2010. Similarly, use of ITNs by children less than five years of age and by pregnant women rose from 4% to 70% and from 4% to 72%, respectively, from 2000 to 2010. The percentage of households owning at least one ITN for every two household members (access) increased from 3% to 39% from 2000 to 2010. To assess the equity of ITN access, ownership and use in Rwanda from 2000 to 2010, data on household wealth and ITNs from Demographic and Health Surveys in 2000, 2005 and 2010 were used to compute Lorenz Concentration Curves and Indices. Concentration Index (C-Index) values range between -1 and 1 with a value of 0 representing perfect equality. Results show drastic improvements in equity of ITN ownership over time (C-Index: 0.35 in 2005 compared to 0.07 in 2010), household ITN access (C-Index: 0.42, 0.12, 0.06 in 2000, 2005 and 2010, respectively), ITN use in children less than five years (C-Index: 0.66, 0.33 and 0.05 in 2000, 2005 and 2010, respectively) and ITN use in pregnant women (C-Index: 0.70, 0.25 and 0.03 in 2000, 2005 and 2010, respectively). Results suggest that ITN distribution programs in Rwanda have achieved increasing equity over time such that by 2010, levels of ITN access, ownership and use were similar across households of all wealth quintiles. This may be due, in part, to the shift in distribution from target populations to mass campaigns.

1250

STRUCTURED SUPERVISION VISITS USING TABLETS FOR IMPROVEMENT OF MALARIA CASE MANAGEMENT IN SENEGAL

Julie I. Thwing[1], William Olander[2], Medoune Ndiop[3], Moustapha Cisse[3], Mame Birame Diouf[4], Alioune B. Gueye[3], Ibrahima Diallo[3], Mady Ba[3]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Independent Consultant, Dakar, Senegal, [3]Senegal National Malaria Control Program, Dakar, Senegal, [4]United States Agency for International Development, Dakar, Senegal

Improving the quality of malaria case management in public health facilities in Senegal is an ongoing challenge, with 32 hospitals, 89 health centers, and 1247 health posts. The National Malaria Control Program (NMCP) instituted health facility supervision visits to improve malaria services, supervising over 800 structures annually. During each visit, a lengthy paper form is completed, which includes rapid diagnostic test technique, consultation observation, register abstraction of case management of uncomplicated and severe malaria, and stock management. Supervisors are chosen from a cadre of health officers who have attended training in malariology conducted by the NMCP. Health personnel and district health teams receive feedback based on a synthesis of the results. However, are not entered electronically and capacity is limited to perform detailed analysis of the data collected. The NMCP piloted the use of Android tablet computers to facilitate improved data collection and analysis of supervision visits. The supervision form was programmed using Open Data Kit, which provided internal data checks, automatically calculated scores, and collected Global Positioning System (GPS) coordinates for each facility. After completion of each round of supervision, data are downloaded at the NMCP and analyzed. The test phase included 4 hospitals, 4 epidemic surveillance sites, 4 health centers and 3 health posts. While none were out of stock of all dose-packs of artemisinin-based combination therapy (ACT), stockouts of the infant dose, the 1-5 year dose , the 6-13 year dose, and the adult dose affected 7%, 40%, 7%, and 70% of facilities, respectively. All facilities had rapid diagnostic tests (RDT) in stock. Data were missing regarding RDT performance in 10% of febrile patients, but an RDT was performed in 95% of the suspect cases for which data were available. Of patients with a positive RDT, 92% were documented to have received an ACT. Of severe cases, 93% of patient < 5 years and 69% of patients ≥5 years were judged to have been managed correctly. During 2014, tablets will be used to conduct supervision visits nationwide, reducing time required to compile reports, and enabling rapid feedback, in-depth analysis of case management, mapping of indicators, and increased capacity to identify and correct deficiencies. This provides a powerful tool for monitoring malaria case management and an evidence base for continuous quality improvement.

1251

INCREASING HUMAN RESOURCES FOR MALARIA PROGRAM IMPLEMENTATION IN LOW RESOURCE SETTING: THE IMPACT OF A MALARIOLOGY COURSE FOR PUBLIC HEALTH PROVIDERS IN SENEGAL

Alioune B. Gueye[1], Medoune Ndiop[1], Julie I. Thwing[2], Mame Birame Diouf[3]

[1]Senegal National Malaria Control Program, Dakar, Senegal, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]United States Agency for International Development, Dakar, Senegal

The Senegal National Malaria Control Program (NMCP) aggressively scaled up malaria control interventions during 2007-2010 and is now moving toward pre-elimination. However, the NMCP did not have the human resource capacity to implement and supervise the interventions, particularly correct case management, throughout the public health system of 32 hospitals, 76 district health centers, 1,247 health posts, 2,162

health huts, and 1992 home-based care providers. In order to address this challenge, the NMCP developed an annual malariology course to train public health personnel, with a focus on district health management teams. Each year, senior and middle management health personnel are invited to attend three and two week residential courses, respectively, in malariology, including planning, implementation, monitoring and evaluation. Since 2008, 50 senior and 65 middle managers have been trained, with technical support from WHO and the school of public health, at a cost of $153 per day for senior managers and $107 per day for middle managers. The NMCP recruits from this pool of trained managers for many activities, including training providers on guidelines for malaria prevention and case management, supervision of over 800 health facilities and their providers at all levels in biannual sessions of 21 days each, and periodic assessment of quality of case management. District health officials trained by the NMCP were crucial to the successful adoption of rapid diagnostic tests, enabling Senegal to test over 85% of suspected cases by the second year of implementation. During 2013, they supervised a total of 2,116 providers, seeing an average of 8 providers per working day. Currently, they are training personnel in the newly revised case management guidelines, including pre-referral treatment with rectal artesunate and treatment of severe disease with parenteral artesunate. The Senegal NMCP has trained a critical mass of district-level managers in malaria, who have facilitated the implementation, monitoring and supervision of malaria control activities in a context of a shortage of human resources, and whose contribution to the success of malaria control efforts in Senegal has been consistent and cannot be underestimated. This approach is recommended for other low resource malaria endemic countries struggling with lack of qualified personnel to implement malaria control efforts.

1252

FACILITATING HARMS DATA CAPTURE BY NON-CLINICIANS UTILIZING A NOVEL DATA COLLECTION TOOL DEVELOPED BY THE ACT CONSORTIUM - RESULTS OF TESTING IN THE FIELD

Cheryl Pace[1], Sham Lal[2], Victoria Ewing[3], Anthony K. Mbonye[4], Richard Ndyomugyenyi[4], David G. Lalloo[1], Dianne J. Terlouw[3]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]Malawi-Liverpool-Wellcome Trust Clinical Programme, Blantyre, Malawi, [4]Ministry of Health, Kampala, Uganda

In recent years, rapid diagnostic tests and enhanced delivery strategies have improved access to efficacious antimalarials by those who need them. As case management improves and preventative measures such as Mass Drug Administration (MDA) are considered in order to reduce both disease burden and transmission, the potential acceptable risk-benefit ratio to end users has shifted; monitoring the safety of these drugs, however, has been slow to rise on the public health agenda. Whilst novel strategies for improving access to antimalarials and disease burden surveillance are being employed, we still rely on the traditional weak, inefficient and in many places virtually non-existent pharmacovigilance system of clinician-led reporting within the context of the conventional healthcare setting. As antimalarials are increasingly being provided via non-conventional routes and by lower-level healthcare workers, the importance of equipping these workers with the appropriate tools to monitor and report on possible drug-related patient-experienced harms becomes paramount. Traditional pharmacovigilance data collection forms are complex and challenging to use by non-clinicians. The ACT Consortium developed data collection tools to allow lower-level healthcare workers to collect high quality harms data within a variety of contexts such as research studies and programmatic, real-life settings. These tools use a pictorial storyboard to convey the need for data collection to a low-literacy level population. A diary captures drug administration and event data in chronological relation to each other with minimal interpretation required by the data collector, thereby making it suitable for use by lower-level healthcare staff. These tools were used and tested by non-clinical data collectors within ACT Consortium projects and preliminary analysis of the results show that the harms data collected are comparable to those collected within the same and similar clinician-led studies.

1253

IMPACT OF SCHOOL-BASED PROGRAM OF MALARIA DIAGNOSIS AND TREATMENT ON SCHOOL ATTENDANCE IN SOUTHERN MALAWI

Katherine E. Halliday[1], Don Mathanga[2], Stefan Witek-McManus[1], Austin Mtali[3], Doreen Ali[4], John Sande[4], Reuben Mwenda[5], Charles Mazinga[6], Tiyese Chimuna[7], Natalie Roschnik[8], Simon Brooker[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Malaria Alert Centre, College of Medicine, Blantyre, Malawi, [3]Save the Children International, Zomba, Malawi, [4]National Malaria Control Program, Lilongwe, Malawi, [5]Health Technical Support Services-Diagnostics, Ministry of Health, Lilongwe, Malawi, [6]Ministry of Education, Science and Technology, Lilongwe, Malawi, [7]Save the Children International, Lilongwe, Malawi, [8]Save the Children International, Washington, DC, United States

Whilst real progress in the goal of education for all has been made in sub-Saharan Africa, evidence indicates children who suffer from ill health are less likely to attend and complete school. Malaria is an important cause of morbidity in school children and a significant contributor to school absenteeism. To address the burden of malaria in this age group, the Malawian National Malaria Control Programme, with support from Save the Children, are currently implementing a programme of school-based malaria case management in Southern Malawi. A cluster randomised trial in 58 schools in Zomba is evaluating the impact of this programme whereby malaria rapid diagnostic tests (mRDTs) and artemisinin-based combination therapies (ACTs) to diagnose and treat uncomplicated malaria have been placed in primary schools, as part of basic first aid kits [Learner Treatment Kits (LTKs)]. Head teachers and two additional teachers in the schools were trained in the use of mRDTs and the additional contents of the kits at a 7-day residential workshop. Twenty nine schools were randomly selected to receive the LTKs and a further twenty nine schools were selected to serve as the control. Baseline findings indicated 60.0% (95% CI: 56.2-63.7%) children in this region were infected with *Plasmodium falciparum*, while the prevalence of anaemia was 32% (95% CI: 29.2-35.5%). We present data from teachers' treatment registers describing uptake of the malaria diagnostic service by learners, including the number of malaria cases diagnosed by teachers. Treatment and referral practices will also be reported. Additionally, we present preliminary results on the impact of LTKs, comprising malaria diagnosis and treatment and basic first aid, on the principal outcome of school attendance. To our knowledge, this is the first instance in which school teachers have been trained to perform mRDTs and provide treatment on the basis of parasitological confirmation. Such a programme could provide a valuable complementary service to facility- and community-based roll out of mRDTs.

1254

THE ECONOMIC VALUE OF THE POST-TREATMENT PROPHYLACTIC EFFECT OF FIRST-LINE ANTIMALARIAL TREATMENTS ACROSS DIFFERENT MALARIA TRANSMISSION SETTINGS

Johannes Pfeil[1], **Yesim Tozan**[2]

[1]University of Heidelberg, Heidelberg, Germany, [2]New York University, New York, NY, United States

Recent trials show that dihydroartemisinin-piperaquine (DHAPQ) is as efficacious and safe as artemether-lumefantrine (AL) in treating uncomplicated childhood malaria and has a longer post-treatment prophylactic (PTP) effect compared to AL, reducing the risk of re-infection in children. In a recent cost-effectiveness analysis, we showed that DHAPQ was superior to AL from both the clinical and economic perspectives for treatment of uncomplicated childhood malaria in high transmission

settings (*in press at Plos One*). From a clinical and economic perspective, the benefits of post-treatment prophylaxis are, however, expected to become more significant with increasing transmission intensity and, conversely, less significant with decreasing transmission intensity. Our aim in this analysis is to assess the economic value of the PTP benefit conferred by DP compared to AL across different transmission settings. We base our analysis on primary clinical outcome data from a multi-centre clinical trial of ACTs that was conducted in Asian (n=998) and African children (n=1,698) in a wide range of malaria transmission settings (data provided by Sigma Tau I.F.R. SpA). Using the Markov model we developed for the previous analysis, simulating the progression of malarial disease and the risk of recurrent malaria, we estimate the mean incremental costs and health outcomes of the two treatment strategies per child over one year from the provider perspective in transmission settings stratified as low, moderate and high. We employ probabilistic sensitivity analysis to account for uncertainty in key model parameters. Our preliminary results show that the economic value of the PTP effect of DHAPQ over AL is significant in moderate to high transmission settings, using maximum manufacturer drug prices for ACTs set by the Global Fund. Our full analysis will report how the extent of this benefit varies by local malaria endemicity and antimalarial drug prices. Our findings should help inform the policy discussions on the choice of optimal malaria treatment strategy across endemic settings.

1255

THE 'PADDY PARADOX' REVISITED: HOW RICE FARMING IMPACTS ON HOUSEHOLD ECONOMIC STATUS AND MALARIA RISK IN EASTERN RWANDA

Alexis Rulisa[1], Fred Kaatera[1], Chantal Ingabire[1], Emmanuel Hakizimana[1], Michele Van Vugt[2], Leon Mutesa[3], Luuk Van Kempen[4]

[1]Rwanda Biomedical Center, Kigali, Rwanda, [2]Academic Medical Center, Amsterdam, Netherlands, [3]University of Rwanda, Huye, Rwanda, [4]Radboud University Nijmegen, Nijmegen, Netherlands

Economic activities may entail negative externalities for public health, which is particularly problematic in poverty-stricken areas. The case of rice farming in eastern Rwanda fits this description, as it provides breeding sites for malaria-infested mosquitoes but at the same time generates cash income and improves nutritional standards locally. We add to the evidence base on the 'paddy paradox' by studying a case in Eastern Rwanda (Ruhuha district of Bugasera province). The study unpacks the impact of rice cultivation on malaria incidence by comparing households that differ in their involvement in rice cultivation and proximity to the marshlands that host the rice fields. To this purpose, a large-scale survey was conducted among more than 4,000 households (comprising 17,000 individuals) in the area from June to December 2013. Data on household demographics, economic status, malaria prevention efforts as well as health-seeking behavior has been collected. All household members have also been screened for malaria parasitemia and anemia, and a malnutrition assessment was carried out for under-five children. In addition, qualitative data was collected through nine focus group discussions. It is shown that rice farming is positively and significantly associated with households' wealth, food security, health insurance status, and protection against malaria. At the same time, it is confirmed that rice farming practices increase the risk of malaria transmission through expanded mosquito populations. Rice fields are the main breeding site in the area. Households located nearby the marshlands where rice is cultivated are the most affected by malaria. For those households who generate income from rice production directly, the income effect dominates, resulting in a lower disease burden from malaria. By contrast, households in communities that are located close to the rice cultivation areas but who do not participate in this economic activity, face a higher malaria burden. Rice farming leads to private benefits in the economic domain, which spills over into the health domain, but at the same time creates a public health risk. As a result, the 'paddy paradox' hypothesis is confirmed at the level of rice-producing households, but rejected at the wider community level. Hence, strategies need to be developed that are able to tap the private benefits of rice cultivation and re-direct these to fund collective action against malaria.

1256

SERUM 8,12-ISO-IPF2α-VI ISOPROSTANE MARKER OF OXIDATIVE DAMAGE AND COGNITION DEFICITS ASSOCIATED WITH CASSAVA CYANOGENIC POISONING

Desire Tshala-Katumbay[1], K. Kambale[2], L. D. Okitundu[2], B. M. Bumoko[2], Michael J. Boivin[3], D. N. Mumba[2], J. J. Tamfum-Muyembe[2], J. P. Banea[2]

[1]Oregon Health and Sciences University, Portland, OR, United States, [2]University of Kinshasa, Kinshasa, Democratic Republic of the Congo, [3]Michigan State University, East Lansing, MI, United States

We sought to determine whether motor (konzo) and cognitive deficits associated with cassava (food) cyanogenic poisoning were associated with high levels of F2-isoprostanes, well-established indicators of oxidative damage. Levels of serum F2-isoprostanes were quantified by LC-MS/MS and anchored to measures of motor proficiency and cognitive performance assessed through BOT-2 or KABC-II neuropsychological testing of 40 Congolese children [21 with konzo and 19 presumably healthy controls, overall mean age (SD): 9.3 (3.2) years]. Cyanogenic exposure was ascertained by levels thiocyanate (SCN) in plasma and urinary. Overall, levels of plasma SCN ranged from 91 to 325 µmol/l or 172 to 1032 µmol/l in plasma or urine, respectively. Levels of isoprostanes (ng/ml) ranged from 01 to 0.8 (Isoprostane-III), 0.8 to 8.3 (total Isoprostane-III), 0.1 to 1.5 (Isoprostane-VI), 2.0 to 9.0 (total Isoprostane-VI), or 0.2 to 1.3 ng/ml (8,12-iso-iPF2α-VI isoprostane). Children with konzo poorly performed both at the BOT-2 and KABC-II testing ($p<0.01$) Within a regression model controlling for age, gender, and other biochemical variables, 8,12-iso-iPF2α-VI isoprostane (ng/ml) was significantly related to overall cognitive performance (Mental Processing Index) on the KABC-II ($\beta = -32.36$ (-51.59 to -13.03 95% CI; $P<0.001$). A regression model including age, gender, motor proficiency impairment, serum albumin and triclyceride levels, and 8,12-iso-iPF2α-VI isoprostane in 20 konzo children explained over 85% of variation in the overall Mental Processing Index, but was not significant in explaining the overall motor proficiency impairment . These findings suggest that brain/behavior injury associated with cassava poisoining is mediated in part by oxidative stress injury. We conclude that 8,12-iso-iPF2α-VI isoprostane is a sensitive biomarker of the neuropathogenic mechanisms mediating brain injury in konzo, and can be used to monitor the impact of interventional trials to prevent or mitigate the neurotoxicity effects of cassava cyanogenic poisoning.

1257

LEAD AND IRON INTERACT TO INCREASE *PLASMODIUM FALCIPARUM* PARASITEMIA IN BENINESE INFANTS

Violeta Moya-Alvarez[1], Michael Osei Mireku[1], Smaila Ouedraogo[2], Manfred Accrombessi[2], Michel Cot[3], Florence Bodeau-Livinec[4]

[1]Institut de Recherche pour le Développement, EHESP, Paris/Benin, France, [2]Institut de Recherche pour le Développement, Benin, France, [3]Institut de Recherche pour le Développement, Paris/Benin, France, [4]Ecole des Hautes Etudes en Santé Publique, Rennes, France

Lead poisoning is a major public health challenge in West Africa. It hinders the correct neurocognitive development of infants and it entails impaired growth and learning disorders as well as kidney damage and anaemia. In Benin over 80% of the children are anemic. Malaria, another major cause of anemia, is the first cause of infant morbidity and mortality. However, the interaction of lead poisoning, anaemia and malaria has not been investigated so far. By analyzing the effect of lead levels on *P. falciparum* parasitemia we aim at unravelling the impact of lead poisoning on malaria episodes among Beninese infants. We have followed 630 infants up to 12 months and we have assessed their health status with regard to malaria,

other parasites, as well as their haematological profile. In addition their blood lead levels have been analyzed at 12 months of age showing a high prevalence of lead poisoning (17% for blood lead level (BLL)≥10 µg/dL). Multivariate models show significant increased *Plasmodium falciparum* parasitemia associated with increased BLL irrespective of iron status. In addition there is a positive significant association of total body iron with *P. falciparum* parasitemia controlling for clinical, demographic and environmental malaria risk factors. The interaction of BLL and total body iron is also significantly associated with *P. falciparum* parasitemia. In conclusion the significant association impact of lead levels on *P. falciparum* parasitemia and the synergistic interaction of iron and lead on *P. falciparum* parasitemia bring up important research and public health questions in a region where malaria and lead poisoning overlap. To our knowledge this effect had not been shown so far and complementary cohort studies are required to confirm its significance. In any case, the synergistic interaction between lead and iron rises up the importance of analyzing further the convenience of iron supplementation for anaemia treatment and prophylaxis, especially in malaria endemic regions with high exposure to lead. The considerable prevalence of lead poisoning addresses the necessity of investigating the sources of lead pollution and analyzing its impact on the neuro-cognitive development of Beninese infants, as well as its impact on anaemia.

1258

QUALITATIVE AND QUANTITATIVE EVALUATION OF IMPROVED STOVE ACCEPTABILITY AND MULTIPLE STOVE USE IN RURAL WESTERN KENYA

Jennifer D. Loo[1], Tamara Pilishvili[1], Bryan Christensen[1], Ibrahim Sadumah[2], Ronald Nyagol[2], Mike Sage[3], Fuyuen Yip[1], Stephanie Schrag[1], Nigel Bruce[4], Debbi Stanistreet[4]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Safe Water and AIDs Project, Kisumu, Kenya, [3]Global Alliance for Clean Cookstoves, Washington, DC, United States, [4]University of Liverpool, Liverpool, United Kingdom

The majority of households in rural Western Kenya cook indoors using open biomass fires, increasing exposure to household air pollution (HAP), which has the potential to negatively impact health. Our study aimed to evaluate the impact of improved cookstove technologies (ICT), compared to the traditional 3-stone open fire, on effectiveness in reducing HAP and acceptability of use in the community. Preliminary findings showed that many households were using additional stoves, in conjunction with the ICT evaluated during the study. This analysis explores factors associated with multiple stove use among study households. We employed mixed methods in a cross-over study design to evaluate 6 different ICT (2 rocket, 1 rocket with chimney, 3 fan-assisted) in households. One ICT was placed in each household for a 2 week period and was intended to be the sole stove used for daily household tasks. Stoves were rotated until each house had used at least 5 different ICT. Households were monitored for 48-hour periods at the end of each 2 week round and quantitative questionnaires, a cooking activity log, qualitative interviews and focus groups were completed. Multiple stove use was defined as any use of additional stoves other than the ICT under evaluation during the 48-hour monitoring period. Of 43 households, 67% (n=29) indicated use of multiple stoves during the study [8 households (19%) at least 25% of the time, 11 (26%) half of the time, 7 (16%) 75% of the time and 3 (7%) all of the study]; 14 (33%) households reported single stove use. Multiple stove use occurred most often (17/38 households, 45%) when the chimney stove was present and least often (9/35, 26%) when rocket stove B was present. Qualitative findings indicate that stove type, ease of stove use, number of people cooked for and type of meal cooked are likely factors associated with multiple stove use. These findings demonstrate the difficulties of conducting field evaluations for ICT and highlight key factors to consider in developing ICT that are acceptable in meeting the daily needs of users.

1259

TRACHOMA CONTROL THROUGH IMPROVED ACCESS TO WATER AND SANITATION

Mariamo Mbofana[1], Sharone Backers[2], **Philip Downs**[3], Brian Chu[4], Rebecca Mann Flueckiger[5], Rebecca Willis[4], Alex Pavluck[4], Anthony Solomon[6]

[1]Ministry of Health Mozambique, Maputo, Mozambique, [2]RTI International, Maputo, Mozambique, [3]RTI International, Washington, DC, United States, [4]Task Force for Global Health, Atlanta, GA, United States, [5]International Trachoma Initiative, Atlanta, GA, United States, [6]World Health Organization, Department of Control of Neglected Tropical Diseases, Geneva, Switzerland

Facial cleanliness is a key activity to prevent blinding trachoma; a disease which has impaired the vision of more than 2 million people worldwide. The SAFE strategy (Surgery, Antibiotic, Facial cleanliness, Environmental improvement) for trachoma elimination is substantially weakened without access to water and sanitation facilities. According to leading water, sanitation, and hygiene (WASH) specialists, 12.7 million people in Mozambique (over half the population) don't have access to safe water or proper sanitation facilities. The relationship between water, sanitation, and trachoma is complex; those who have easy access to water and latrines may or may not have less active trachoma. To determine whether access to water and latrines at the household level is associated with greater access to functioning hand-washing facilities and soap, the national trachoma control program in Mozambique reviewed results of recent trachoma baseline prevalence mapping, which includes data on basic WASH indicators. Households with water close to the home (water source in yard) were more likely to have access to a hand-washing facility (P<.001). Households with access to a private latrine were more likely to have access to a hand-washing facility, P<.001, RR=1.20 (1.19-1.22), have water available at the hand-washing facility, P<.001, RR=1.15 (1.14-1.16), and soap available at the hand washing facility, P<.001, RR=1.06 (1.05-1.06) compared to households with only access to a public latrine. In households that have access to safe water and latrines, but lack access to hand-washing facilities and soap, programs should identify the barriers to adopting hand and face washing behavior and modify their Behavior Change Communication (BCC) strategies as appropriate. Promotion of private latrine construction may complement BCC strategies and should be targeted in areas with high prevalence of trachoma.

1260

HYDROLOGICAL DRIVERS OF TYPHOID TRANSMISSION IN KIBERA, AN URBAN SLUM IN NAIROBI, KENYA

Adam N. Akullian[1], Eric Ng'eno[2], Alastair I. Matheson[1], Daniel Macharia[2], Leornard Cosmas[2], Judd L. Walson[1], Joel M. Montgomery[2]

[1]University of Washington, Seattle, WA, United States, [2]Centers for Disease Control and Prevention - Kenya, Nairobi, Kenya

Typhoid fever is a systemic enteric disease caused by *Salmonella* vars *typhi* and *paratyphi*. Typhoid fever occurs primarily in densely populated urban areas with poor sanitation infrastructure. The primary route of transmission is thought to be via direct contact with individuals who shed the bacteria in their stool. However, recent evidence from Asia suggests that indirect transmission via contaminated surface water may also play a role in the spread of disease. Using data from a large population-based infectious disease surveillance system operated by the Kenya Medical Research Institute/US Centers for Disease Control (KEMRI/CDC), we mapped the spatial pattern of typhoid fever risk in Kibera, an urban slum in Nairobi Kenya, with an extremely high population density (70,000 individuals/sq km). Cases were defined as individuals with fever and positive blood culture for *S. typhi*. Controls were selected randomly from the population-based cohort to estimate the spatial distribution of the underlying population at risk, and were matched to cases on age, gender, and date of diagnosis. We used a spatial modeling framework to map the

geographic distribution of typhoid fever cases and to test whether any significant spatial patterns could be explained by variations in topography and surface-water-accumulation. The greatest risk of typhoid fever was among those living in the lowest-elevation areas where surface-water flow accumulates (p = 0.01). Our results support indirect environmental transmission of typhoid fever in resource-limited settings. Interventions targeted at reducing typhoid fever transmission (e.g., improvements in sanitation and hygiene, and typhoid vaccination) in upstream areas of typhoid-endemic regions may indirectly benefit residents in downstream areas, who are at increased risk of exposure to *S. typhi* from both immediate and upstream sources of contamination.

1261

CERAMIC WATER FILTERS AND REDUCING THE BURDEN OF DIARRHEAL DISEASE IN INFANTS - WESTERN KENYA, 2013

Jamae Morris[1], Chandra Schneeberger[1], Peter Jaron[2], Fenny Moke[2], Jane Juma[2], John Ochieng[2], Richard Omore[2], Tracy Ayers[1], Jennifer Murphy[1], Jeffery Priest[1], Lihua Xiao[1], Barry Fields[3], Joel Montgomery[3], John Vulule[4], Eric Mintz[1], Vincent Hill[1], Ciara O'Reilly[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Kenya Medical Research Institute/Centers for Disease Control and Prevention, Kisumu, Kenya, [3]Centers for Disease Control and Prevention-Kenya, Nairobi, Kenya, [4]Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya

Unsafe drinking water consumption is a risk factor for diarrhea, a leading cause of death in sub-Saharan African children. Ceramic water filters (CWFs) remove or inactivate waterborne diarrheal pathogens in drinking water through size exclusion and silver exposure. We examined the effectiveness of CWFs to improve drinking water quality and prevent infantile diarrhea in rural western Kenya. A randomized**,** controlled intervention trial was conducted among 240 households with infants 4-10 months old. Each household was randomized into an intervention or control group with or without CWFs, respectively. Trained interviewers performed a baseline survey and visited households weekly for 26 rounds to document recent onset of diarrhea, respiratory infection, and febrile illness in infants. Source and filtered water samples were tested to monitor *Escherichia coli* concentrations, measured as most probable number (MPN). Person-time incidence rates were calculated per 100 person-weeks of observation. Households reported using surface water (36.3%), public taps (29.2%), or rainwater (17.1%) as their primary drinking water sources. Self-reported filter use among intervention households was 99.6% across weeks observed. Compared with the control group, intervention households reported fewer diarrheal episodes (7.6 vs. 8.9, p=0.1) and fewer health facility visits for diarrhea (1.2 vs. 2.2, p<0.01). The incidence of respiratory infection (1.3 vs. 1.1, p=0.61) and febrile illness (4.1 vs. 4.1, p=0.9) remained similar. *E. coli* were detected in 93% of source water samples (median concentration 512 MPN/100mL; range 10 - $1.4x10^4$ MPN/100mL) and in only 29% of filtered water samples (median concentration 7.4 MPN/100mL; range <1.0 - >2420 MPN/100mL). Households using CWFs had improved water quality and reported lower incidence and significantly fewer health facility visits for diarrhea in infants.

1262

WATER ACCESS, QUALITY AND USE IN TEN HEALTHCARE FACILITIES IN HONDURAS AND GHANA

Katharine Robb, Marisa Gallegos, Ledor Igboh, Samantha Lie-Tjauw, Christine Moe

Emory University, Atlanta, GA, United States

In the developing world, it is estimated that 46% of health facilities have access to an improved water source. The 2015 Sustainable Development Goals include a provision to "provide universal access to safe drinking water in health centers." In order to meet this goal, the majority of health facilities in the developing world will need to gain access to an improved water source. However, once access to an improved source is obtained, there remain significant barriers to ensure sustainable water access, quality, and use. From 2012-2014 the Center for Global Safe Water at Emory conducted an assessment of water access, quality and use in 10 district-level hospitals in Honduras and Ghana. Water quality testing, observations and interviews were conducted at each site. All hospitals evaluated had access to improved water sources and on-site treatment systems. Despite this, barriers such as intermittent water and power supplies, wards without piped connections, broken taps, and limited water access points for patients and visitors reduced water access in the hospital. Hospitals increased water access through the use of cisterns and bucket taps. Hospitals spent significant funds to increase water access by purchasing water from tanker trucks and buying bottled water. Methods to improve access often resulted in decreased water quality and increased costs. In over 300 samples tested for E. coli, 77% of samples in Honduras and 61% in Ghana met international drinking water standards. Samples from piped taps were 4 times more likely to meet drinking water standards compared to samples from bucket taps (p=0.0256). Despite variable quality, tap water was used for a variety of drinking, hygiene and medical purposes. In Honduras, 24% of staff reported using tap water for drinking versus 5% of staff in Ghana. Tap water is used for reconstituting and giving medications by 23% of clinical staff in Honduras and 14% in Ghana. While 19% of staff in Ghana use tap water for wound care, no staff in Honduras reported using tap water for wound care. A common barrier to the use of safe water is lack of knowledge about the quality of water from various sources within the hospital. In conclusion, despite improved water sources at healthcare facilities, there exist persistent challenges to consistent safe water access and use. Attaining universal and sustained safe water access and use will require assessment of barriers and the development of mitigation strategies.

1263

EOSINOPHILS DRIVE CELLULAR INNATE IMMUNITY ACTIVATION TO CONTROL HELMINTH LARVAE MIGRATION AND PROMOTE LUNG TISSUE REMODELING BY A TNF-DEPENDENT PATHWAY DURING *ASCARIS* INFECTION

Pedro H. Gazzinelli-Guimaraes[1], Ana Clara Gazzinelli-Guimaraes[1], Vitor L. Mati[1], Fernando S. Barbosa[1], Emilia Araujo[1], Paula Vieira[2], Deborah Negrao-Correa[1], Claudia M. Carneiro[2], Peter Hotez[3], Daniella C. Bartholomeu[1], Lilian Bueno[1], Ricardo T. Fujiwara[1]

[1]Federal University of Minas Gerais, Belo Horizonte, Brazil, [2]Federal University of Ouro Preto, Ouro Preto, Brazil, [3]Sabin Vaccine Institute and Texas Children's Hospital Center for Vaccine Development, National School of Tropical Medicine, Baylor College of Medicine, Houston, TX, United States

While eosinophils have been associated with resistance to helminthic infections, the most important findings related to these cells are based on the peripheral eosinophilia observed in nematode-infected individuals and in the host intestinal mucosal response. In contrast, the role of eosinophils during hepatic-tracheal migrations of nematodes larvae (as frequently occurs in many human nematode infections) is not well understood. In this study eosinophils were evaluated during early *Ascaris* larval infections in wild-type (WT) BALB/c mice compared with an eosinophil-deficient mice model (ΔdblGATA). The absence of host eosinophils resulted in: 1) an increase in the number of *Ascaris* larvae migrating through the liver and lung; 2) a parallel reductions in the pulmonary inflammatory response, with reduced inflammatory infiltrated cells in the parenchymal lung tissue and bronchoalveolar lavage fluid; 3) a decrease in the levels of IL-6 and myeloperoxidase (MPO) produced by related-innate immunity cells during the peak of larvae migration; and 4) a decrease in the production of eosinophil-dependent TNF and eosinophil peroxidase (EPO) in the lungs, with impairments in pulmonary tissue remodeling. Taken together, this study suggest that eosinophils have key roles in both controlling the number of tissue-migrating *Ascaris* larvae and promoting associated airway inflammation through activation of host innate immunity pathways.

Simultaneously, eosinophils promote pulmonary tissue remodeling by EPO and TNF-dependent pathways during larval *Ascaris* sp. infections. These findings suggest an innovative hypothesis on the evolution of eosinophils in the mammalian host-parasite relationship.

1264

HUMANIZED NOD-SCID IL-2RΓNULL (NSG) MICE: RESPONSE TO INFECTION WITH *STRONGYLOIDES STERCORALIS*

Sandra Bonne-Annee[1], John Patton[2], Jessica Hess[2], Tim Manser[2], James B. Lok[3], Thomas J. Nolan[3], Raja Vuyyuru[2], David Abraham[2]

[1]National Institutes of Health, Bethesda, MD, United States, [2]Thomas Jefferson University, Philadelphia, PA, United States, [3]University of Pennsylvania, Philadelphia, PA, United States

Strongyloides stercoralis is a parasitic nematode that infects humans, non-human primates and dogs. Immunocompetent mice will allow infections to persist for up to two weeks with little development beyond the larval stages. Both innate and adaptive immune responses control the infection in mice, with roles for complement component C3, eosinophils, neutrophils and macrophages in the protective innate immune response. Furthermore, CD4+ T cells, TH2 cytokines and the production of parasite-specific IgM and IgG are central to the adaptive immune response in mice. The goal of this study was to characterize *S. stercoralis* infection in the immunodeficient NOD-scid IL-2Rγnull (NSG) and humanized NSG (HIS) mice. Following exposure to the infective stage larvae of *S. stercoralis*, NSG mice supported larval and adult stages of the parasite. Naïve NSG mice had similar levels of C3 when compared to naïve C57BL/6J mice, which retained its ability to collaborate with C57BL/6J effector cells in killing the parasites *in vitro*. However, NSG mice demonstrated an absence of eosinophils and similar neutrophil numbers when compared to C57BL/6J mice. To determine if HIS mice were also susceptible to *S. stercoralis* infection, HIS mice were generated by engrafting NSG mice with human hematopoietic stem cells. HIS mice were susceptible to the infection, although these mice harbored 40% fewer adult parasites than NSG mice. Analysis of HIS mice following infection revealed the presence of human IgM and IgG demonstrating that HIS mice can establish a parasite-specific humoral response. We conclude from these studies that NSG mice are susceptible to the complete *S. stercoralis* life cycle. This may be explained by an absence of eosinophils or a deficit in the function of other effector cells. HIS mice had reduced parasite levels, which suggests that the human adaptive response is playing a role in the control of the parasite. These studies demonstrate that NSG and HIS mice are useful tools for dissecting the immune response of both mice and humans to *S. stercoralis*.

1265

EFFECTS OF MATERNAL GEOHELMINTH INFECTIONS ON THE DEVELOPMENT OF ATOPY, ECZEMA AND WHEEZE DURING THE FIRST THREE YEARS OF LIFE: FINDINGS FROM THE ECUAVIDA BIRTH COHORT

Philip J. Cooper[1], Martha E. Chico[2], Maritza Vaca[2], Leila Amorim[3], Carlos A. Sandoval[2], David P. Strachan[4], Laura C. Rodrigues[5], Mauricio L. Barreto[3]

[1]St. George's University of London, London, United Kingdom, [2]Laboratorio de Investigaciones FEPIS, Quininde, Ecuador, [3]Universidade Federal da Bahia, Salvador, Brazil, [4]St George's University of London, London, United Kingdom, [5]London School of Hygiene & Tropical Medicine, London, United Kingdom

To investigate the effects of maternal geohelminth infections on the development of atopy and allergic disease in early childhood, we analysed data from a birth cohort in a rural District of Esmeraldas Province. Ecuador. A total of 2,404 newborns were recruited in a public hospital serving the District of Quininde and were evaluated at 13, 24, and 36 months of age for eczema and wheeze. Skin prick test (SPT) reactivity to aero and food allergens was measured at 36 months. A single stool sample was collected from the mother in the third trimester of pregnancy and examined for the presence of geohelminth infections using a combination of microscopic methods including Kato-Katz and formol-ether concentration methods. Data was analyzed by multivariate logistic regression. We had complete follow-up data for 2,082 (86.6%) children through to 3 years of age. Geohelminth infections were detected in 46.1% of mothers and were predominantly infections with *Ascaris lumbricoides* (28.0% of mothers) and *Trichuris trichiura* (28.7%). The prevalence of outcomes in children by 3 years was: any episode of eczema (17.5%) and wheeze (26.0%), and SPT (17.2%). Maternal geohelminth infections were associated with an increased risk of eczema by 3 years of age (OR 1.28, 95% CI 1.02-1.61) but with a decreased prevalence of SPT (OR 0.82, 95% CI 0.61-1.00). No association was observed with wheeze (OR 1.01, 95% CI 0.82-1.25). Our data show that maternal geohelminths, in an Ecuadorian population where *Ascaris* and *Trichuris* are the predominant infections, are associated with an increased risk of eczema but with a reduced prevalence of SPT in early childhood.

1266

SYSTEMIC CYTOKINE PRODUCTION, GEOHELMINTH INFECTION AND NEURODEVELOPMENTAL OUTCOMES IN BANGLADESHI INFANTS

Nona M. Jiang[1], Fahmida Tofail[2], Shannon N. Moonah[1], Rebecca J. Scharf[1], Mami Taniuchi[1], Jennie Z. Ma[1], Priya Duggal[3], Genevieve L. Wojcik[3], Eric R. Houpt[1], Shihab U. Sobuz[2], Jena D. Hamadani[2], Eduardo Azziz-Baumgartner[4], Rashidul Haque[2], William A. Petri, Jr.[1]

[1]University of Virginia, Charlottesville, VA, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [3]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [4]Centers for Disease Control and Prevention, Atlanta, GA, United States

An estimated one-third of children under 5 in low- and middle-income countries do not reach their full developmental potential. We recently published that systemic inflammation is linked to the neurodevelopment of children from a slum community in Dhaka, Bangladesh. An interesting finding from our study was that elevated levels of the Th2 cytokine IL-4 in 6-month sera were associated with higher cognitive scores. This finding raises the question of what was driving higher levels of IL-4 in children. Here we tested in the same cohort of Bangladeshi children for factors that could explain why some children had elevated levels of IL-4. Since environmental factors such as helminth infections drive a Th2 response in the host, we tested monthly surveillance stools for the first 6 months of life for the presence of intestinal helminths using multiplex PCR assays. We found that nearly 40% of children were infected with at least one intestinal helminth, with *Ascaris lumbricoides* and *Trichuris trichiura* being the most prevalent at 25% and 16%, respectively. *Ascaris lumbricoides* infection was associated with elevated levels of IL-4 in 6-month sera (p=0.02). Additionally, *Trichuris trichiura* infection was associated with higher cognitive, language, and motor scores on the Bayley Scales of Infant and Toddler Development III at 30 months of age (all p<0.05). We are validating our findings on systemic inflammation and neurodevelopment in a second cohort of children in Dhaka, and are testing for the impact of a SNP in the promoter region of IL-4 (C-589T) that has been shown to influence IL-4 production. The results from these additional studies will be presented. In conclusion, IL-4 and *Trichuris trichiura* infection were associated with better developmental test scores. In addition, elevated levels of IL-4 can partly be explained by helminth infection in this cohort of infants from a low-income setting. Elucidating the cause of elevated IL-4 would greatly enhance our ability to modulate levels of systemic IL-4, which may promote healthy cognitive development in at-risk children.

1267

THE GENOME AND TRANSCRIPTOME OF THE ZOONOTIC HOOKWORM *ANCYLOSTOMA CEYLANICUM* REVEAL INFECTION-SPECIFIC GENE FAMILIES

Erich M. Schwarz[1], Yan Hu[2], Igor Antoshechkin[3], Melanie M. Miller[4], Paul W. Sternberg[3], Raffi V. Aroian[2]

[1]Cornell University, Ithaca, NY, United States, [2]University of Massachusetts Medical School, Worcester, MA, United States, [3]California Institute of Technology, Pasadena, CA, United States, [4]University of California San Diego, La Jolla, CA, United States

Hookworms infect over 500 million people, stunting and impoverishing them. Sequencing hookworm genomes, and finding which genes they express during infection, should help devise new drugs or vaccines against hookworm. Unlike other hookworms, *Ancylostoma ceylanicum* infects both humans and other mammals, providing a laboratory model for hookworm disease. We determined an *A. ceylanicum* genome sequence of 313 Mb, with transcriptomic data throughout infection showing expression of 30,738 genes. ~900 genes were upregulated during early infection *in vivo*, including ASPRs, a cryptic subfamily of Activation-associated Secreted Proteins (ASPs). ASPR genes are also present in the related intestinal parasites *Necator americanus*, *Oesophagostomum dentatum*, and *Heligmosomoides bakeri*, but not the trichostrongylid parasite *Haemonchus contortus*. Genes downregulated during early infection include ion channels and G protein coupled receptors; this downregulation is observed in both parasitic and free-living nematodes. Another novel family of genes are upregulated as larvae develop to the L4 stage and migrate into the intestine; this family has homologs in *N. americanus*, *H. contortus*, and *Angiostrongylus cantonensis*, and its products are predicted to be nonclassically secreted. Still later in infection, as *A. ceylanicum* matures to young adulthood and begins drinking host blood, C-lectin genes are strongly upregulated, some of whose products resemble vertebrate more than nematode lectins. These findings provide new drug and vaccine targets, and should elucidate hookworm pathogenesis.

1268

DETECTION OF GASTROINTESTINAL PARASITES BY MULTI-PARALLEL QUANTITATIVE REAL-TIME PCR AND ASSOCIATIONS WITH GROWTH DELAY IN EARLY CHILDHOOD: FINDINGS FROM A BIRTH COHORT IN RURAL ECUADOR

Rojelio Mejia[1], Andrea Arévalo[2], Yosselin Vicuña[2], Pablo Espinosa[2], Martha E. Chico[2], Maritza Vaca[2], Carlos Sandoval[2], Peter J. Hotez[1], Philip J. Cooper[2]

[1]National School of Tropical Medicine, Houston, TX, United States, [2]Fundacion Ecuatoriana para la Investigacion en Salud, Quito, Ecuador

Gastrointestinal (GI) parasites may have important influences on growth and nutrition in childhood. Previous studies investigating the effects of parasite infections on growth have tended to use poorly sensitive microscopic-based assays. To investigate the effects of single and multiple parasite infections on growth in young children we analyzed data from a birth cohort study in Ecuador, correlating GI parasite affects on anthropometric measures. Stool samples from a random sample of 400 children in the cohort were collected at 13, 24, and 36 months of age and analyzed using our rapid, high throughput multi-parallel quantitative real-time PCR (qPCR) for the 8 most common gastrointestinal parasite pathogens including the helminths, *Ascaris lumbricoides, Ancylostoma duodenale, Necator americanus, Strongyloides stercoralis, Trichuris trichiura* and protozoa, *Cryptosporidium parvum, Entamoeba histolytica* and *Giardia lamblia.* Each child had anthropometric data collected at the same time points including height, weight, head and abdominal circumference. The qPCR detected increased prevalence of infections for *Ascaris* at 13, 24, and 36 months (6.8%, 12.9%, and 15.5%, respectively). Similar results were seen for *Giardia* (31.5%, 44.5%, and 51.6%, respectively) and other parasites. Furthermore, children that were infected at a previous time point tended to be infected at subsequent observation times with higher concentrations of parasite DNA for *Ascaris* and *Giardia* (fg/μL, $p < 0.05$) For all parasites, qPCR was more sensitive than standard microscopic methods. GI parasite infections were associated with growth delays for all anthropometric parameters by comparison with WHO growth curves; growth of abdominal circumference was less in the infected group (1.5 cm) compared to the non-infected group (4 cm)($p = 0.0054$). In conclusion, we have deployed a high throughput, rapid, quantitative molecular based system that has improved diagnostic accuracy compared to stool microscopy. Our data also indicate that GI parasite infections may affect growth during the first years of life.

1269

THE EFFECT OF DEWORMING TIMING AND FREQUENCY ON GROWTH IN EARLY PRESCHOOL-AGE CHILDREN: RESULTS OF A RANDOMIZED-CONTROLLED TRIAL OF MEBENDAZOLE IN ONE TO TWO-YEAR OLD CHILDREN IN THE PERUVIAN AMAZON

Serene A. Joseph[1], Martin Casapia[2], Theresa W. Gyorkos[1]

[1]McGill University, Montreal, QC, Canada, [2]Asociacion Civil Selva Amazonica, Iquitos, Peru

Children under two years of age are in the most critical window for growth and development. As mobility increases, this time also coincides with first exposure to soil-transmitted helminth (STH) infections in tropical environments. WHO recommends deworming as of 12 months in endemic areas; however, the optimal timing and frequency have been understudied in this age group. Many countries still exclude children 12-23 months in deworming programs. We conducted a randomized-controlled trial of deworming (500mg single-dose mebendazole) in 12 and 13 month-old children in Iquitos, an STH-endemic area of the Peruvian Amazon. A total of 1760 children were enrolled from September 2011 to June 2012 at 12 participating health centres. Children were randomly allocated to one of four groups: 1) deworming at 12 months of age and placebo at 18 months of age; 2) placebo at 12 months of age and deworming at 18 months of age; 3) deworming at 12 and 18 months of age; or 4) placebo at 12 and 18 months of age (i.e. control group). Participants were followed up to 24 months of age to assess the benefit of deworming on the main outcome of weight gain. Results were analyzed with an intention-to-treat approach. A total of 1563 children (88.8%) attended their 24 month visit. STH prevalence rose from 12.2% at 12 months to over 40% at 24 months. Mean weight gain (kg) between 12 and 24 months was: Group 1): 2.05 (±0.7); Group 2): 1.94 (±0.8); Group 3): 2.04 (±0.7); and Group 4): 2.00 (±0.7). There was a statistically significant improvement in weight gain in those receiving deworming once at 12 months, compared to those receiving deworming once at 18 months (p=0.028). No difference was detected between those receiving deworming once at 12 months vs. twice at 12 and 18 months (p=0.88). Results remained significant when adjusting for baseline characteristics. Additional analyses were performed to take into account clustering, multiple testing, missing data and compliance. Overall, our results indicate that deworming, provided once-yearly at 12 months of age, has important benefits on growth in early preschool-age children. These results contribute to the evidence-base on deworming policy in over 120 STH-endemic countries worldwide. Emphasis should be placed on translating results into practice, such that children in this vulnerable age group are targeted with the most cost-effective, integrated interventions to reduce health and nutritional burdens.

1270

RE-EMERGENCE OF DENGUE IN SOUTH TEXAS, 2013

Dana L. Thomas

Centers for Disease Control and Prevention, San Juan, PR, United States

Sporadic dengue outbreaks have occurred in south Texas for over 30 years. In 2013, during a dengue epidemic in northern Mexico, 17 suspected dengue cases were identified in two border counties in south Texas during July-October. To characterize the outbreak, Texas Department of State Health Services and CDC implemented enhanced surveillance by: 1) reviewing medical records at eight hospitals for dengue-like illness; 2) performing RT-PCR on serum specimens from suspected dengue cases previously tested by anti-dengue virus (DENV) IgM ELISA at commercial laboratories during October-December; and 3) conducting interviews with laboratory-positive dengue case-patients and offering household members dengue diagnostic testing by RT-PCR and IgM ELISA. During 2013, clinicians in south Texas requested dengue diagnostic testing for 246 patients. Of these, 54 (22%) were laboratory-positive: 32 (59%) by IgM ELISA, 15 (28%) by RT-PCR, and 7 (13%) by both. Of 84 specimens that were negative by IgM ELISA at commercial laboratories and further tested by RT-PCR, 15 (18%) were positive. Of 22 cases positive by RT-PCR, DENV-1 was detected in 19 (86%) and DENV-3 was detected in 3 (14%). Of all laboratory-positive dengue case-patients, 26 (48%) had not left Texas in the 14 days before illness onset, and 20 (38%) reported recent travel to Mexico. Of 22 dengue case-patients' households investigated, 5 (23%) had at least one additional household member with evidence of recent DENV infection without travel history. During a dengue outbreak associated with an epidemic in northern Mexico, enhanced surveillance in south Texas identified the largest number of locally acquired dengue cases ever detected. Dengue diagnostic testing should include both IgM ELISA and RT-PCR as evidenced by the high rate of false negatives with anti-DENV IgM testing alone, due to the number of patients who submit specimens during the acute phase of their infection. Since the burden of dengue is expected to continue in south Texas, dengue surveillance and laboratory capacity should continue to be improved.

1271

LARGE BURDEN OF DENGUE AND WEST NILE VIRUS TRANSMISSION IN COASTAL KENYA

A. Desiree LaBeaud[1], Tamara Banda[1], Crystal Teng[1], Chelsea Heimbaugh[1], Eric Muchiri[2], Peter Mungai[2], Francis M. Mutuku[3], Julie Brichard[1], Ginny Gildengorin[1], Erin M. Borland[4], Ann Powers[4], Uriel Kitron[5], Charles H. King[6]

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

Dengue virus (DENV) and West Nile virus (WNV) are endemic to most regions of the world, but accurate prevalence data are lacking in Sub-Saharan Africa. The objective of this study was to measure the burden of DENV and WNV exposure in coastal Kenya and link it to demographics and other risk factors. Demographic and exposure questionnaires were administered to 1,013 participants recruited from two coastal villages, Milalani (55% of total) and Nganja (45%), in 2009. Sera were screened for flavivirus exposure using a commercial DENV IgG ELISA and then confirmed with plaque reduction neutralization tests (PRNT). Chi square, Fisher exact test, t tests and logistic models were used to determine variables that were associated with seropositivity. 343 (35%; 95% CI 32-38%) participants were seropositive (aged 1-87 years, mean 37 years). Ten percent (95% CI 8-14%) of children were seropositive vs. 53% (95% CI 49%-58%) of adults. Of 297 PRNT confirmed positives, 203 samples (68% of positives, 20% of total) were DENV, 49 samples (16% of positives, 5% of total) were WNV, and 45 samples (15% of positives, 5% of total) had high PRNT titers for both DENV and WNV. Age was significantly associated with seropositivity (OR 1.07 per year, 95% C.I. 1.06-1.08). Males, adults who owned a radio or television, and those with schistosomiasis, malaria, or *Trichuris* were less likely to be seropositive ($p<0.05$). A greater proportion of DENV- and WNV-confirmed participants resided in Milalani, though the association with Village was not significant. Flavivirus exposure, particularly DENV, is very common in coastal Kenya, with more than half of adults exposed. Adults and females are more likely to be seropositive, whereas those with parasitic infections are less likely. Interepidemic transmission is suggested by many DENV and WNV seropositive children. The high flavivirus burden documented suggests that DENV and WNV are important causes of disease in coastal Kenya, but limited surveillance, clinical overlap with malaria and other viruses, and limited diagnostics contribute to under-reporting.

1272

VARIABILITY IN DENGUE TITER ESTIMATES FROM PLAQUE REDUCTION NEUTRALIZATION TESTS POSES A CHALLENGE TO EPIDEMIOLOGICAL STUDIES AND VACCINE DEVELOPMENT

Henrik Salje[1], Isabel Rodríguez-Barraquer[1], Kaitlin Rainwater-Lovett[1], Ananda Nisalak[2], Butsaya Thaisomboonsuk[2], Stephen J. Thomas[3], Stefan Fernandez[2], Richard G. Jarman[3], In-Kyu Yoon[2], Derek A. Cummings[1]

[1]Johns Hopkins School of Public Health, Baltimore, MD, United States, [2]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [3]Walter Reed, Silver Spring, MD, United States

Accurate determination of neutralization antibody titers supports epidemiological studies of dengue virus transmission and vaccine trials. Neutralization titers measured using the plaque reduction neutralization test (PRNT) are believed to provide a key measure of immunity to dengue viruses, however, the assay's variability is poorly understood, making it difficult to interpret the significance of any assay reading. In addition, there is limited standardization of the PRNT cut-point or statistical model used to estimate titers across laboratories, with little understanding of the optimum approach. We used repeated assays on the same two pools of serum using five different viruses (2,319 assays) to characterize the variability in the technique under identical experimental conditions. We also assessed the performance of multiple statistical models to interpolate continuous values of neutralization titer from discrete measurements from serial dilutions and identified the optimal PRNT cut-point for the assay. We found that the variance in plaque reductions for individual dilutions was 0.016, equivalent to a 95% confidence interval of 0.45 - 0.95 for an observed plaque reduction of 0.7. We identified PRNT75 as the optimum cut-point with a variance of 0.025 (log10 scale), indicating a titer reading of 1:500 had 95% confidence intervals of 1:240 - 1:1000 (2.70±0.31 on a log10 scale). The choice of statistical model was not important for the calculation of relative titers, however, cloglog regression out-performed alternatives where absolute titers are of interest. Finally, we estimated that only 0.7% of assays would falsely detect a four-fold difference in titers between acute and convalescent sera where no true difference exists. Estimating and reporting assay uncertainty will aid the interpretation of individual titers. Laboratories should perform a small number of repeat assays to generate their own variability estimates. These could be used to calculate confidence intervals for all reported titers and allow benchmarking of assay performance.

1273

CROSS-REACTIVITY TO HETEROLOGOUS DENV TYPES INCREASES IN THE YEARS FOLLOWING PRIMARY INFECTION AND IS MAINTAINED FOLLOWING SECOND INFECTION IN A PEDIATRIC DENGUE COHORT STUDY IN NICARAGUA

Leah Katzelnick[1], Magelda Montoya[2], Lionel Gresh[3], Angel Balmaseda[4], Eva Harris[2]

[1]Centre for Pathogen Evolution, University of Cambridge, Cambridge, United Kingdom, [2]Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States, [3]Sustainable Sciences Institute, Managua, Nicaragua, [4]Laboratorio Nacional de Virología, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud, Managua, Nicaragua

The four dengue virus (DENV) serotypes infect an estimated 390 million individuals each year. Following a first DENV infection, the neutralizing antibody response is thought to become increasingly specific to the infecting serotype, and over time, is expected to remain only to the infecting serotype. After secondary infection, neutralization of all serotypes is thought to be relatively balanced and persist over time. However, these hypotheses have not been tested rigorously in a longitudinal cohort. We used reporter viral particles (RVPs) to measure changes in the type-specificity of neutralizing antibody responses to all four DENV serotypes in healthy annual samples from a cohort of Nicaraguan children for 1-6 years after their natural first, second, and, in some cases, third infections. Post-infection neutralizing titers were ranked from highest to lowest in the year after infection, and fold-difference in neutralization between the best-neutralized serotype and each heterologous serotype was measured every year until subsequent infection. As expected, the trajectories of neutralizing responses after infection varied by individual in magnitude and degree of cross-reactivity between serotypes. However, when first-infection neutralizing antibody responses were analyzed as a group, increasing cross-reactivity was observed over time. Specifically, the difference between the best and second-best neutralized serotypes decreased over time, with a mean decline of 1.3-fold/year ($p<0.01$), from an average difference of 8.5-fold in the first year after infection ($p<0.001$). This effect remained significant when serotype, year, and infection outcome were taken into account. Indeed, while the titer to the infecting type did not change significantly over time, the second-best neutralized serotype and third-best neutralized serotype increased by 1.3 fold/year ($p<0.01$) and 1.2 fold/year ($p<0.05$), respectively. When neutralizing antibody responses were analyzed in the same subjects following subsequent second infections, cross-reactivity between serotypes did not change significantly over time, although the magnitude of titers to all four serotypes decreased over time. We find that children living in Nicaragua become more cross-reactive to heterologous DENV serotypes over time following first infection and maintain cross-reactive responses following second infection.

1274

RELATIVE INCIDENCE OF ADULT AND PEDIATRIC DENGUE VIRUS INFECTION IN A PROSPECTIVE LONGITUDINAL COHORT IN THE PHILIPPINES

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

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

Dengue is the most common mosquito-borne viral infection globally. In Asia where dengue has been hyperendemic for many years, the average age of dengue has been increasing. However, dengue incidence and clinical spectrum have not been as well characterized in adults as in children. In order to determine the incidence of dengue virus (DENV) infection in adults compared to children and the relative proportion of subclinical versus symptomatic infections, a longitudinal prospective cohort of approximately 1000 subjects aged ≥6 months was initiated in Cebu, Philippines in March 2012 and underwent community-based active surveillance for febrile episodes. Acute and 3-week convalescent blood samples were obtained and tested by DENV RT-PCR/nested PCR (acute samples) and DENV IgM/IgG EIA (acute/convalescent samples). Enrollment and 12-month follow up samples were tested by DENV hemagglutination inhibition (HAI) to identify subclinical seroconversion. During one year of surveillance, the annual incidence of total and symptomatic DENV infection in the cohort was 8.5% and 1.5%, respectively. The total and symptomatic incidence in the 6 month-5 year old age group was 11.0% and 2.5%; 6-15 years was 15.3% and 4.4%; 16-30 years was 7.4% and 0.5%; 31-50 years was 4.2% and 0%; >50 years was 4.4% and 0%. DENV-1 was the predominant serotype. The total and symptomatic incidence among 139 subjects with negative DENV HAI at enrollment was 10.1% and 2.9%; among 32 subjects with one positive DENV HAI serotype was 31% and 9.4%; among 682 subjects with ≥2 positive HAI serotypes was 7.3% and 0.7%. Fifty-five percent of subjects ≤15 years old had multitypic HAI whereas 96% of those >15 years old were multitypic. Our results indicate that DENV infection is less frequent and less likely to be symptomatic in adults than children in a hyperendemic area, but much of this effect is due to preceding immune status. DENV infection in adults may become more symptomatic if force of infection decreases due, for example, to future pediatric vaccination programs.

1275

DENGUE VIRUS-SPECIFIC T CELL RESPONSES IN THE GENERAL POPULATION OF NICARAGUA VARY AS A FUNCTION OF THE INFECTING SEROTYPE AND ARE DOMINATED BY HLAB35-RESTRICTED EPITOPES

Daniela Weiskopf[1], Cristhiam Cerpas[2], Rukie de Alwis[1], Michael A. Angelo[1], Derek J. Bangs[1], Ravi V. Kolla[1], John Sidney[1], Bjoern Peters[1], Lionel Gresh[3], Angel Balmaseda[2], Eva Harris[4], Alessandro Sette[1]

[1]La Jolla Institute for Allergy and Immunology, La Jolla, CA, United States, [2]Laboratorio Nacional de Virología, Managua, Nicaragua, [3]Sustainable Sciences Institute, Managua, Nicaragua, [4]Division of Infectious Diseases and Vaccinology, Berkeley, CA, United States

Infections with any of the four dengue virus serotypes (DENV1-4) occur with high incidence in more than 100 countries around the world, accounting for as many as 390 million infections each year. All four DENV serotypes have circulated extensively in Nicaragua in recent years, and as a result, the adult population has generally been exposed to all four serotypes. To assess the T cell response against all four DENV serotypes, we tested predicted motifs in an *ex vivo* ELISPOT assay for their ability to induce an IFNγ response in HLA-matched peripheral blood mononuclear cells (PBMC) of 124 Nicaraguan Red Cross blood donors from the general adult population of Managua, Nicaragua. This proteome-wide screen identified a total of 314 CD8+ T cell epitopes across all 10 DENV proteins (C, M, E, NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5). Interestingly, we observed different immunodominance patterns of targeted DENV antigens depending on the serotype. DENV3-specific responses equally targeted structural and nonstructural (NS) proteins while DENV1-, DENV2- and DENV4-specific responding epitopes were found to disproportionately (>90%) target NS proteins, especially NS3, NS4B and NS5. We found 30% of the epitopes identified were novel, while 70% were also found in a previous study of Sri Lankan blood donors. Additionally, we observed a striking dominance of HLA B-restricted responses in general and of HLA B*35 in particular, both in terms of breadth as well as magnitude of the DENV-specific CD8+ T cell responses. Interestingly, this allele has been associated with protection from disease in a different population study in Malaysia. We found that the majority of responses were produced by T cells displaying an effector memory phenotype (TEMRA and TEM). In terms of cytokine expression patterns, the majority of cells were double-positive for IFNγ and TNFα, indicating a multifunctional phenotype. Our results provide new insights into HLA-restricted T cell responses against all four DENV serotypes, which are of relevance for both vaccine design and the identification of robust correlates of protection in natural immunity.

1276

EXPLORING THE CELLULAR METABOLOME AS A GATEWAY TO TARGET DENGUE VIRUS REPLICATION IN THE MOSQUITO VECTOR

Nunya Chotiwan[1], Irma Sanchez-Vargus[1], Jeffrey M. Grabowski[2], Amber Hopf-jannasch[3], Victoria Hedrick[3], Erik Gough[3], Ernesto Nakayasu[3], Devika Sirohi[4], Catherine A. Hill[5], Richard J. Kuhn[6], Rushika Perera[1]

[1]Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO, United States, [2]Department of Biological Sciences, Department of Entomology, Purdue University, West Lafayette, IN, United States, [3]Bindley Bioscience Center, Purdue University, West Lafayette, IN, United States, [4]Department of Biological Sciences, Purdue University, West Lafayette, IN, United States, [5]Department of Entomology and Bindley Bioscience Center, Purdue University, West Lafayette, IN, United States, [6]Department of Biological Sciences and Bindley Bioscience Center, Purdue University, West Lafayette, IN, United States

The cellular metabolome plays a significant role in the life cycle of enveloped arthropod-borne viruses. For instance, in both the human host and mosquito vector, flaviviruses significantly modify lipid metabolism to enter and exit from cells, as well as to assemble membrane targeted replication factories for efficient viral RNA replication. Additionally, cellular metabolic changes assist in diverting or evading host antiviral defenses. Using technical advances in high-resolution mass spectrometry we have profiled the metabolome of dengue virus (DENV) infected mosquitoes and analyzed the metabolic changes that occur in the salivary glands and midgut tissues during the time course of infection. These studies were carried out in parallel to analysis of the human metabolome, also during infection with DENV. Our results indicated that DENV infection altered the expression of lipids that had the capacity to change the physical properties of the membrane bilayer such as curvature, permeability, and the recruitment and assembly of protein complexes in the membrane. Several of the identified molecules also functioned as bioactive messengers that controlled signaling and membrane trafficking pathways in the cells. Through these efforts we have generated a metabolomic fingerprint of DENV infection within the human host and its mosquito vector, *Aedes aegypti*. We are now exploring the mechanism of how DENV exploits these metabolic pathways for its replication and are evaluating these pathways as novel avenues for the development of antivirals that could target virus replication in both the human and vector hosts. Through these efforts we have also facilitated data linkage between two NIAID Biological Resource Centers, (the virus pathogen resource (ViPR) and VectorBase (VB) to provide these data to the greater scientific community.

1277

TPL2-DEPENDENT INDUCTION OF IL-10 IN HUMAN ALTERNATIVELY ACTIVATED MACROPHAGES FOLLOWING MYCOBACTERIAL INFECTION: INSIGHTS INTO THE HELMINTH/MYCOBACTERIAL INTERFACE

Soumya Chatterjee, Emily E. van Seventer, Joseph Kubofcik, Roshanak Tolouei Semnani, Thomas B. Nutman

National Institutes of Health/National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States

Filarial and other tissue invasive helminth infections are associated with an early IL-4 driven expansion of Th2 cells that in turn drive the expansion of alternatively activated macrophages (AAMφs). In light of the geographic superimposition of tuberculosis and filarial infection we sought to understand how mycobacteria are handled in the context of helminth-induced AAMφ. Using human AAMφs generated *in vitro* from human monocytes by IL-4 and comparing them to LPS and IFN-γ generated classically macrophages (CAMφs) both infected with mycobacteria (BCG), we were first able to demonstrate that AAMφs were more susceptible to infection with BCG than were CAMφs (p=0.02) and this susceptibility was associated with increased IL-10 production, a cytokine known to enhance immune evasion of mycobacteria by impairing macrophage phagolysozome killing and antigen presentation. Not only did mycobacteria increase the production of IL-10 in AAMφs and not in CAMφs; (p=0.017) but we were also able to show that tumor progression locus 2 (TPL2), an upstream activator of extracellular signal related kinases (ERKs) acting through STAT3, itself induces IL-10 production. To explore the relationship between TPL2 and IL-10 in our *in vitro* BCG AAMφ model, we generated both CAMφs and AAMφs, exposed them to BCG at an MOI of 5 and examined TPL2 and its effects. Using qRT-PCR as well as by Western blot, we found increased baseline induction of TPL2 in CAMφs but not AAMφs (p=0.04). Post BCG infection, however, TPL2 levels were increased in AAMφs only (p=0.03). AAMφs (but not CAMφs) showed significantly diminished IL-10 production following the addition of the TPL2 kinase inhibitor ($C_{21}H_{14}ClFN_6$) (IC_{50}= $500x10^{-9}$ M) (p=0.001) BCG infection. These data show that AAMφs (commonly generated in human helminth infection) but not CAMφs activate the positive feedback loop for IL-10 regulation by induction of TPL2, suggesting a mechanism by which IL-10 production is increased in response to mycobacterial infection.

1278

CREATING AN EVIDENCE-BASED AND CLINICALLY-RELEVANT THRESHOLD FOR TB-ASSOCIATED CATASTROPHIC COSTS: A COHORT STUDY, PERU

Tom Wingfield[1], Delia Boccia[2], Marco Tovar[3], Arquímedes Gavino[4], Karine Zevallos[4], Rosario Montoya[4], Knut Lonnroth[5], Carlton A. Evans[1]

[1]Imperial College London, United Kingdom and Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Department of Infectious Disease Epidemiology, London School of Hygiene & Tropical Medicine, London, United Kingdom, [3]Universidad Peruana Cayetano Heredia and IPSYD, Lima, Peru, [4]IPSYD, Lima, Peru, [5]Policy Strategy and Innovations, Stop TB Department, World Health Organization, Geneva, Switzerland

Even when tuberculosis (TB) treatment is free, hidden costs incurred by TB-affected households may worsen poverty and health. Extreme TB-associated costs are termed 'catastrophic' but are poorly defined. We studied TB-affected households' hidden costs and their association with adverse TB outcome to create a clinically-relevant definition of catastrophic costs, against which we compared existing thresholds. From 2002-2009, TB patients (n=876, 11% with multi-drug resistant TB) were recruited to a prospective cohort study in shantytowns in Lima, Peru. Patients were interviewed prior to and every 2-4 weeks throughout treatment recording TB-related costs. Costs were expressed as a proportion of that household's annual income. Adverse TB outcome was defined as: death, abandonment or treatment failure, or TB recurrence. 23% (166/725) of patients had adverse TB outcomes. Total costs ≥20% of household annual income were defined as catastrophic because this threshold was most strongly associated with adverse TB outcome. Catastrophic costs were incurred by 345 households (39%). Adverse TB outcome was independently associated with multi-drug resistant TB (OR=8.4, p<0.001), previous TB (OR=2.1, p=0.005), and catastrophic costs (OR=1.7, p=0.01). Adjusted population attributable fraction of adverse outcomes explained by catastrophic costs was 18% (95%CI=6.9-28), similar to MDR TB (20%, 95%CI=14-25). Sensitivity analyses demonstrated that existing catastrophic costs thresholds (greater or equal to 10% or 15% of household annual income) were not associated with adverse TB outcome in our setting. In conclusion, despite free TB care, having TB disease was expensive for impoverished TB patients in Peru to afford. Incurring higher relative costs was associated with adverse TB outcome. Population attributable fractions implied that MDR TB and catastrophic costs had a similar association with adverse TB outcome. As opposed to existing catastrophic costs thresholds, our novel threshold was found to be clinically-relevant in our setting. Our results show that TB is a socioeconomic as well as infectious problem. Tuberculosis control interventions should address both the economic and clinical aspects of TB and policy makers should consider this new evidence-based and clinically-relevant catastrophic costs definition.

1279

INCREASED TUBERCULOSIS INFECTION IN MEN IN PERUVIAN SHANTYTOWNS DESPITE A DECADE OF DECREASING TUBERCULOSIS DISEASE

Leonardo Martinez[1], Sinead C. Isaacson[1], Carlton A. Evans[2], Richard A. Oberhelman[1], Lilia Cabrera[3], Mayuko Saito[4], Robert H. Gilman[5]

[1]Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States, [2]Wellcome Trust Centre for Clinical Tropical Medicine, Department of Infectious Diseases and Immunity, Imperial College London Hammersmith Hospital Campus, London, United Kingdom, [3]Asociación Benéfica Proyectos en Informática, Salud, Medicina y Agricultura, Lima, Peru, [4]Tohoku University Graduate School of Medicine, Sendai, Japan, [5]Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Changes in tuberculosis (TB) disease incidence rates do not reliably indicate TB control because they are prone to ascertainment bias. In contrast, tuberculin skin test (TST) surveys determine the prevalence of TB infection and allow changes in the community annual risk of infection (ARI) to be estimated objectively. We aimed to analyze changes in ARI from 2000 to 2011 in Pampas de San Juan de Miraflores, a Peruvian shantytown. We conducted 3 tuberculin surveys in different years: in 2000 (n=1056), 2005 (n=103), and lastly in 2011 (n=428). Randomly selected shantytown residents were included and had a TST if not pregnant or never received TB treatment. In 2000 and 2011, participants age ≥5 years were included but in 2005 only those age ≥15 years were available. Participants were stratified into youths (5-14 years) or adults (≥15 years). In 2000, the mean age was 18 (IQR 10-32) years and increased in 2005 and 2011 to 29 (IQR 22-38) and 31 (IQR 15-48) years ($p<0.0001$). To account for age differences we standardized the 2000 ARI rate to the 2011 study age distribution when comparing overall rates. Age-standardized ARI in 2000 was 1.9% (95% CI: 1.8, 2.2), similar to actual rates in 2005 and 2011: 2.4% (95% CI: 1.9, 3.0) and 2.2% (95% CI: 1.9, 2.9). Over time, ARI increased among adult males (2.0% [95% CI: 1.7, 2.4] in 2000; 3.1% [95% CI: 2.0, 4.7] in 2005; 3.0% [95% CI: 2.3, 3.6] in 2011) but was similar for adult females (1.5% [95% CI: 1.3, 1.8] in 2000; 2.0% [95% CI: 1.4, 2.8] in 2005; 2.1% [95% CI: 1.7, 2.6] in 2011). Among youths, there were no differences for males (1.2% [95% CI: 0.8, 1.6] in 2000; 1.4% [95% CI: 0.6, 2.3] in 2011) or females (1.4% [95% CI: 1.0, 1.8] in 2000; 1.8% [95% CI: 0.9, 2.8] in 2011) over time. Thus, despite decreasing rates of diagnosed TB disease in this shantytown, transmission causing TB infection was frequent and, from 2000 to 2011, increased significantly in adult men. Consequently, by 2011, adult males were at significantly greater risk of infection than the rest of the population and should be targeted by TB control interventions.

1280

HIGH PREVALENCE OF *PNEUMOCYSTIS JIROVECII* INFECTIONS AMONG MOZAMBICAN CHILDREN <5 YEARS OF AGE ADMITTED TO HOSPITAL WITH SUSPECTED PNEUMONIA

Quique Bassat[1], Miguel Lanaspa[1], Lola Madrid[1], Sónia Machevo[1], Enrique J. Calderón[2], Anna Roca[1], Pedro L. Alonso[1]

[1]Centro de Investigação em Saúde da Manhiça, Manhiça, Mozambique, [2]Instituto de Biomedicina de Sevilla and CIBER de Epidemiología y Salud Pública, Virgen del Rocío University Hospital, Spain

Pneumonia remains the main cause of pediatric mortality in the world. We aimed to assess the specific prevalence of *Pneumocystis jirovecii* (PJ) infections among children <5 years of age admitted to a rural Mozambican hospital with pneumonia, in an area of high HIV prevalence. Methods: As part of an etiology of pediatric pneumonia study, we recruited during 12 months 835 pediatric patients. Collection of standardized clinical data, chest X-rays and screening of nasopharyngeal aspirate (NPA) samples with PCR for 12 respiratory viruses were routinely performed, together with tests for invasive bacterial infection (IBI), malaria, and HIV. Investigations on PJ infection were performed on all NPA samples using a tri-sequential PCR strategy, assessing two multicopy mitochondrial genes (mtLSU y mtSSU) and a third unicopy one, linked to sulfa drug resistance (DHPS). 77/835 (9.2%) of the patients tested positive for at least one of the PJ genes. 32.5% (25/77) patients showed triple (mtLSU, mtSSU and DHPS) gene positivity, while further 41.6% (32/77) showed double (any combination of the three markers) positivity. Twenty (26.0%) further cases tested solely positive for mtLSU. Median age of PCP patients was 3.9 months (IQR 3.1-12.4). Only 30/77 (39.0%) of the confirmed PCP cases had a clinical picture of probable Pneumocystis jiroveci pneumonia (PCP). HIV co-infection was confirmed in 47.8% of the patients with PCP (22/46) for whom HIV results were available. Surprisingly, 16.7% (11/66) of those patients with a valid blood culture result had a concomitant IBI (6 cases of S. pneumoniae, and 5 other bacteria). Viral co-infection was frequent (36/76; 47.4%), being rhinovirus, adenovirus and human metapneumovirus the three commonest viruses found. 5 patients (6.7%) showed also positive *P. falciparum* parasitemias. 15 PCP-infected patients died during admission, yielding a case fatality rate (CFR) of 19.5%, significantly superior to that for non-PCP infections (8.8%; p=0.003). Further 5 PCP patients died at home within the first 21 days post discharge. PCP is a highly prevalent infection among Mozambican infants admitted with severe pneumonia and carries an unacceptably high risk of death, coexisting with other common pediatric infections. The true burden of pediatric PCP in Sub-Saharan Africa needs to be recognized, particularly in the context of the HIV pandemic, and measures to prevent and adequately manage it put in place urgently.

1281

MYCOBACTERIUM TUBERCULOSIS INFECTION INDUCES PERSISTENT NON-RESOLVING INFLAMMATION

Christine A. King[1], Serene John[2], John Kenneth[2], Saurabh Mehta[3]

[1]SUNY Upstate Medical University, Syracuse, NY, United States, [2]St. John's Research Institute, Bangalore, India, [3]Cornell University, Ithaca, NY, United States

Infection with *Mycobacterium tuberculosis* (MTB) is accompanied by an intense inflammatory response thought to be directly related to pathogenesis. Currently, we have little understanding of this inflammatory reaction in individuals and whether it resolves in response to curative treatment. To determine the systemic inflammatory status of individuals we compared the peripheral blood inflammatory gene expression profile of 10 patients with active MTB, latent MTB infection, and cured (for more than six months) MTB, to healthy controls by quantitative PCR in India. Patients with active MTB had dramatically different inflammatory profiles as compared to latent MTB. Patients with active MTB demonstrated greater mRNA levels of potent pro-inflammatory IL-1β and neutrophil chemotactic factors CXCL1 and IL-8, while patients with latent MTB infections had higher levels of monocyte/macrophage activation and chemotactic mediators including IP30, CD14, CXCL3, CCL2 and CCL8 suggesting a switch from a neutrophil centered response to a monocyte/macrophage tailored response. Furthermore, several of these key factors including CD14, IP30, CCL2 and CCL8 remained elevated in cured patients. Our results suggest that MTB infection induces long-term persistent inflammation in the human host in the absence of active infection. Chronic inflammation is widely recognized as a potent driver of many diseases and our data suggests that a significant portion of the population, particularly in high-burden settings such as India, may remain at risk for inflammation-mediated complications even after successful treatment for MTB infection.

1282

LACK OF ASSOCIATION BETWEEN PARASITIC INFECTIONS AND TUBERCULIN SKIN TEST POSITIVITY IN REFUGEES

Mucho M. Mizinduko[1], Elizabeth D. Barnett[2], Anita M. Loughlin[2], Laura F. White[1], Natasha S. Hochberg[1]

[1]Boston University School of Public Health, Boston, MA, United States, [2]Boston University School of Medicine, Boston, MA, United States

Mycobacterium tuberculosis (Mtb) and parasites often co-infect the same person. Immunologic responses to helminth infections may blunt control of mycobacteria, and epidemiologic data suggest an association between helminth infections and tuberculosis disease. Our objective was to determine whether there is an association between parasite infections and positive tuberculin skin tests (TSTs). We reviewed records of patients seen at the Boston Medical Center Refugee Health Assessment Program from 1999-2012. We evaluated demographic characteristics and results of TSTs, stool ova and parasite examinations, complete blood counts with differential, and serologic testing for helminths (done if eosinophilia was found) and looked for an association between TST results and parasite infections. We used multivariate logistic regression models to control for possible confounders (gender, age, WHO region of birth compared to Africa, and protozoal or helminth infection depending on the model). Among 7230 participants, 3843 (53%) were male, mean age was 25 years (range 1-88), and 3355 (46.4%) had positive TSTs. Individuals with positive TSTs were older (mean age 29.9 vs. 21.7 years; p< 0.0001) and more likely to be male (OR = 1.35; 95%CI, 1.23, 1.49). Helminth infections were found in 393 (5.4%) including *Trichuris trichiura* (132/393; 33.6%), *Strongyloides stercoralis* (89; 22.6%), and *Schistosoma* infections (79; 20%). Among 2473 (34%) with protozoal infections, *Blastocystis* spp was found in 1986 (80%). TST positivity was not associated with helminth infections (adjusted OR [aOR] = 1.14; 95%CI, 0.92, 1.41) or protozoal infection (aOR = 1.08; 95%CI, 0.97, 1.20). We found no association between parasitic infections and TST positivity. Unmeasured confounders (HIV infection, poverty, malnutrition, etc.), undetected parasites, pre-departure parasite treatment and other reasons for false negative TSTs may have obscured an effect. More sensitive methods for Mtb detection and a study of the effect of individual parasite species on TSTs are needed to confirm this lack of association.

1283

CHALLENGES OF DETECTING RESISTANCE TO FIRST AND SECOND LINE ANTI-TUBERCULOSIS DRUGS IN SOUTHWESTERN UGANDA

Patrick Orikiriza[1], Becky Tibenderana[2], Mark J. Siedner[3], Frederick Byarugaba[2], Christopher C. Moore[4], Emily E. Evans[4], Maryline Bonnet[5], Anne-Laure Page[5], Joel Bazira[2], Yap Boum, II[1]

[1]Epicentre, Mbarara; Mbarara University of Science and Technology, Mbarara, Uganda, [2]Mbarara University of Science and Technology, Mbarara, Uganda, [3]Harvard Medical School/Massachusetts General Hospital, Boston, MA, United States, [4]University of Virginia, Charlottesville, VA, United States, [5]Epicentre, Paris, France

There are limited data on the drug susceptibility of tuberculosis (TB) in southwestern Uganda. We assessed the proportion of first- and second-line drug resistance in culture-positive tuberculosis specimens from treatment-naïve suspects in southwestern Uganda to guide regional empiric recommendations on multidrug-resistant tuberculosis (MDR-TB). We collected sputum samples for smear microscopy and culture on mycobacterium growth indicator tubes (MGIT) and Lowenstein Jensen (LJ) media from tuberculosis suspects with no prior TB treatment at Mbarara Regional Referral Hospital from February 2009 to February 2013. We tested archived specimens for isoniazid and rifampicin resistance using the MTBDR*plus* assay and GeneXpert. A subsample of isolates selected randomly for geographic variability was also tested with the MTBDR*sl* assay. The resistant isolates were tested further using sequencing and MGIT. Specimens were collected from 190 TB suspects residing within 23 districts of southwestern Uganda, of whom 69% were male, the median age was 33 years (26-43), and the HIV prevalence was 80/190 (42%). No isolates (0%) were rifampicin-resistant and only 1/190 (0.5%) was isoniazid-resistant (0% overall proportion of MDR-TB). Among 92 isolates tested for second-line drug resistance, 71 (77%) had interpretable results, of which 7/71 (10%), 3/71 (4.2%) and 0 (0%) were resistant to fluoroquinolone using MTBDR*sl,* sequencing, and MGIT respectively. None of the isolates were resistant to aminoglycosides, cyclic peptides, or ethambutol. We found no MDR-TB and no resistance to ethambutol or injectables among treatment naïve TB suspects in southwestern Uganda. However, the discrepancy in the fluoroquinolone resistance results of by the three approved methods makes diagnosis difficult and requires establishment of an optimum global second line testing strategy.

1284

ASTMH PERU: THINKING GLOBALLY AND ACTING LOCALLY TO DISSEMINATE GLOBAL HEALTH RESEARCH RESULTS AND TRAIN YOUNG SCIENTISTS

A. Roxana Lescano[1], Daniel G. Bausch[1], Cesar Cabezas[2], Kyle Petersen[1], Karen Goraleski[3], Claudio F. Lanata[1], Alan J. Magill[4], Eduardo Gotuzzo[5], Vilma Bejar[6], Sofia Gonzalez[7], Andres G. Lescano[1]

[1]U.S. Naval Medical Research Unit - 6, Callao, Peru, [2]Instituto Nacional de Salud, Lima, Peru, [3]American Society of Tropical Medicine and Hygiene, Deerfield, IL, United States, [4]Bill & Melinda Gates Foundation, Seattle, WA, United States, [5]Instituto de Medicina Tropical Alexander von Humboldt, Lima, Peru, [6]Instituto de Medicina Tropical Daniel A. Carrion, Callao, Peru, [7]Sociedad Peruana de Enfermedades Infecciosas y Tropicales, Lima, Peru

The Annual Meeting of the American Society of Tropical Medicine and Hygiene (ASTMH) convenes thousands of scientists from around the world to the United States to exchange advances in global health research. Although Peru has had a major presence at the meeting with over 30 abstracts per year in the last decade, the findings are paradoxically less available to many Peruvian scientists, especially scientists in training, for whom financial and logistical barriers often prevent travel to the United States. Thus, an annual local satellite meeting, ASTMH Peru, was established in Lima, as an avenue for dissemination of scientific information and implementation of research results into health policies. Six local and international established leaders in infectious disease research launched ASTMH Peru in 2011. Only abstracts presented at the previous Annual Meeting are included in ASTMH Peru, no call for new abstracts. In addition to the oral and poster sessions, there are keynote presentations by ASTMH leaders on subjects particularly relevant to Peru, including identifying local funding sources, writing scientific manuscripts and grant proposals, and strategies to implement research findings. Primary topics covered are malaria, cysticercosis, dengue fever, leishmania and diarrhea. ASTMH Peru is 100% funded by Peruvian collaborations with registration fees of $28 for professionals and $18 for students. Remaining funds support partial scholarships for young Peruvian scientists to attend the next U.S, meeting if they have an accepted oral presentation. Between 2011 and 2014, the number of posters increased from 45 to 74; oral presentations from 11 to 17, focusing on malaria, cysticercosis, dengue, leishmania and diarrhea. The number of attendees grew from 205 to 388. An important new segment is the support to informed decision making by local authorities. All sessions were heavily attended. Peruvian members in ASTMH also increased from 68 to 101. Six Peruvian scientists have attended the US meeting supported by ASTMH Peru in the last four years. ASTMH Peru constitutes a timely, low cost, and sustainable mechanism for the exchange of high quality scientific knowledge led by local research leaders bridging the Northern and Southern hemispheres together, with a special focus on the training of young scientists.

1285

MECHANICAL, AUTOMATIC INTRAVENOUS VOLUME REGULATOR FOR RESOURCE-LIMITED SETTINGS

Kamal Shah, Erica Skerrett, Matthew Nojoomi, Thor Walker, Kelley Maynard, Michael Pan, Bailey Flynn, Melissa Yuan, Elizabeth Molyneux, Ann Saterbak, Maria Oden, Rebecca Richards-Kortum

Rice University, Houston, TX, United States

We have developed an automatic mechanical volume regulator for IV therapy in the developing world, where 1.4 million children die annually to dehydration caused by diarrhea, malaria, and dengue hemorrhagic fever. These deaths are preventable with IV therapy; however, children who are treated with IV therapy are at risk of overhydration as due to chronic understaffing and high cost of standard volume regulators such as infusion pumps, which require consumables and electricity. Because the risks of overhydration include death, clinicians use oral rehydration for treatment; however, severe cases of dehydration require IV therapy. The IV volume regulator we designed costs less than $80 and does not require electricity. It employs a lever arm with a movable counterweight (similar to a physician's scale) to incrementally dispense IV fluid. A volume indicator slides along the lever arm and allows selection of target volumes in increments of 50 mL. The change in angle of the lever arm as the IV bag drains activates a spring clamp to kink the IV tubing, stopping fluid flow. Performance was assessed in the lab by delivering target volumes of 50 to 850 mL in increments of 50 mL, five flow rates of 20 to 4000 mL/hr, and four initial IV bag volumes between 200 and 1000 mL (n=5 each; n=170 overall). Usability was quantified with a system usability survey (SUS) by 33 nurses, doctors, and medical students at Queen Elizabeth Central Hospital in Blantyre, Malawi. For all parameters, the mean and median residuals were significantly less than 25 mL, and the maximum residual was 30 mL. After training for less than 15 min, Malawian clinicians set up the device within 79.5±31.5 sec. Participants reported a SUS score of 84.4±11.1, which is greater than the 70 threshold for an acceptable product. These promising results will guide the design of a clinical trial evaluating the field accuracy of this device in summer 2014. By enabling clinicians to provide children life-saving IV fluids, this device may potentially prevent over-hydration in resource-limited settings.

1286

GENOME-WIDE ANALYSIS REVEALED THAT *PLASMODIUM FALCIPARUM*-DRIVEN SELECTIVE FORCES MAY HAVE INDUCED HIGH FREQUENCY OF HLA ALLELES ASSOCIATED WITH PODOCONIOSIS

Fasil Tekola-Ayele[1], Adebowale Adeyemo[1], Elena Hailu[2], Chris Finan[3], Abraham Aseffa[2], Gail Davey[4], Melanie Newport[4], Charles Rotimi[5]

[1]National Institutes of Health, Bethesda, MD, United States, [2]Armauer Hansen Research Institute, Addis Ababa, Ethiopia, [3]Wellcome Trust Sanger Institute, Cambridge, United Kingdom, [4]Brighton and Sussex Medical School, Brighton, United Kingdom, [5]National Institutes of Health, Bethesda, MD, United States

Podoconiosis is geochemical elephantiasis of the lower legs among barefoot individuals with long-term exposure to red clay soils. Globally, more than 4 million people are affected. A genome-wide association study conducted by our group in Ethiopia showed that genetic variants in the HLA class II loci confer susceptibility to podoconiosis. Subsequent HLA typing confirmed that among others, HLA-DQB1*02 is a risk allele and HLA-DRB1*13 is a protective allele to podoconiosis. Interestingly, these alleles have been shown to be associated with protection against P.falciparum malaria. It is estimated that three-quarters of the landmass of Ethiopia is malarious predisposing over two-thirds of the population to malaria. In the present study we aimed to investigate possible selective forces that induced high frequency of the HLA alleles associated with susceptibility to podoconiosis. First, we conducted genome-wide analysis of 464,642 single nucleotide polymorphisms (SNPs) comparing ethnic Wolaita Ethiopians (n=120) with 11 population groups from Africa, Europe, and Asia with aim to identify signatures of recent positive selection. We found that HLA loci showing the strongest genome-wide association with podoconiosis were under strong selective pressure in the Ethiopian population, but not in the others. Next, using data from our own cohort and publicly available HLA database, we compared the distribution of DRB1*13 and DQB1*02 alleles in three Ethiopian population groups (Wolaita, Amhara and Oromo) that form 64% of the total population of the country with that of other Sub-Saharan African population groups. We found that the Ethiopian ethnic groups had the highest frequencies of DRB1*13 compared with other populations in Sub-Saharan Africa. Previous studies showed that the DRB1*13-DQB1*05 haplotype was protective against severe malaria in the Gambian population and DRB1*13 was protective against persistent hepatitis B infection. We also found that the Ethiopian population groups had the third highest frequency of DQB1*02 following Burkina Faso's Fulani and Central African Republic's Aka Pygmy. The Fulani closely share the distribution of HLA alleles with the Amhara and Oromo of Ethiopia, and mount stronger humoral immune response against malaria. Together, these data suggest that strong pathogen-driven selective forces induced the high frequency of the risk variants for podoconiosis.

1287

IMPROVING MALARIA SURVEILLANCE THROUGH USE OF MOBILE TECHNOLOGY IN MAINLAND TANZANIA: FINDINGS FROM A PILOT STUDY

Zainab Alidina[1], Oresto Munishi[1], Anold Mutafungwa[1], Elibariki Mwakapeje[2], Ismail Ismail Koleleni[3], Renata Mandike[4], Frank Chacky[4], Winfred Mwafongo[4], Mwendwa Mwenesi[2], Naomi Kaspar[5], Lynn Paxton[6], Mahdi Ramsan[1], Jeremiah Ngondi[1]

[1]Research Triangle Institute, Dar es Salaam, United Republic of Tanzania, [2]Ministry of Health and Social Welfare, Dar es Salaam, United Republic of Tanzania, [3]University of Dar es Salaam, Dar es Salaam, United Republic of Tanzania, [4]National Malaria Control Program, Dar es Salaam, United Republic of Tanzania, [5]President's Malaria Initiative/United States Agency for International Development, Dar es Salaam, United Republic of Tanzania, [6]Malaria Branch and President's Malaria Initiative, Centers for Disease Control and Prevention, Dar es Salaam, United Republic of Tanzania

The Integrated Disease Surveillance and Response (IDSR) system, a paper based system that reports public health surveillance and response data from the health facility level in Tanzania was implemented nationwide in 2002. A mobile phone based electronic system (eIDSR) was created in 2012 to replace the paper based system (paper-IDSR) to increase efficiency and completeness of reporting. A four-week pilot of the eIDSR system took place in Temeke District in Dar es Salaam in November 2013. This study aimed to explore the change in reporting and completeness of surveillance data reporting following the change from the paper-IDSR to the eIDSR system. A total of 67 (64% out of 104 eligible) health facilities participated in the eIDSR pilot following training. For the duration of the pilot, the paper-IDSR and eIDSR system worked concurrently. Data were collected at the district level for the paper-IDSR and through an internet-based database for eIDSR. A data quality assessment was conducted in January 2014 to compare timeliness and quality of data between the two systems. Preliminary findings indicate that 70% of weekly reports were submitted on time through the eIDSR compared to 78% of timely reports via the paper-IDSR system; this is due to a discrepancy in how the paper-IDSR and eIDSR define timeliness (defined by paper-IDSR as Wednesday and eIDSR as Monday 3 pm for the previous week data). Initial analysis indicates that when the same cut off time (Monday) is used for both systems, timeliness in eIDSR is substantially faster than paper-IDSR. All health facilities reported complete data through the eIDSR system while 84% reported complete data through the paper-IDSR system. The paper-IDSR system required dedicated staff to travel from the health facility to the district medical officer to deliver weekly reports. The cost of travel and person hours lost to deliver reports is completely eliminated in the eIDSR system. This

pilot shows that eIDSR improved timeliness of weekly reporting and data completeness. Implementation of eIDSR should be considered in all regions in the Mainland Tanzania to improve surveillance of infectious diseases.

1288

HYBRID STUDY DESIGN FOR COMPLEX INTERNATIONAL FIELD TRIALS: CONDUCTING OBSERVATIONAL RESEARCH ON A RANDOMIZED CLINICAL TRIAL PLATFORM TO ENSURE HIGHEST QUALITY STANDARDS IN SCIENTIFIC DISCOVERY FOR GLOBAL HEALTH

E. Ross Colgate[1], Beth Kirkpatrick[1], Josyf Mychaleckyj[2], Uma Nayak[2], Rashidul Haque[3], Dorothy Dickson[1], Marya Carmolli[1], Jennie Ma[2], Masud Alam[3], Sean Diehl[1], Fardusi Qadri[3], William Petri[2]

[1]University of Vermont College of Medicine, Burlington, VT, United States, [2]University of Virginia, Charlottesville, VA, United States, [3]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

Performing interventional Randomized Clinical Trials (RCT) according to strict standards is time consuming, expensive, and often designed to answer a single question about the impact of an intervention. Infrastructure development for this work is extensive to ensure subject protection, preserve trial endpoints, minimize bias, and promote generalizability of results. Most sponsors or funding agencies are increasingly interested in enhancing the value of this time, cost and infrastructure, as well as investing in innovative research around challenging problems in global health. In large and complex field settings, innovation, the rapid integration of new data and exploratory analyses may be impeded by overly rigid research designs, including standard RCT models. A new Hybrid Study Design (HSD) that combines the strengths and ameliorates the weaknesses of RCT and observational study designs may be a more robust model for maximum translational impact. The Hybrid Study Design ensures the rigor of clinical trials to safeguard data quality and subject protection, while allowing for discovery, particularly in rapidly changing fields where new knowledge needs to be tested, confirmed and applied quickly to improve health outcomes. We report on our experience using a HSD in an urban slum setting in Bangladesh to examine the role of Environmental Enteropathy, a poorly characterized disorder of the small intestine, in oral vaccine underperformance through the PROVIDE Study. With a 2x2 factorial design and two vaccine interventions, the PROVIDE study combined the ethical, regulatory, and analytic structure of a RCT with the flexibility required to successfully undertake cutting-edge research in the developing world. The rationale for the HSD will be presented and lessons learned from applying this new model in a birth cohort of 700 infants with two years of follow-up. We propose the HSD as a useful model for research in which an interventional component or non-inferiority question is added to exploratory or descriptive work, particularly in developing world settings.

1289

INCREASED EQUITY IN MALARIA CONTROL INTERVENTIONS IN MALAWI FROM 2000 TO 2012

Jui Shah, Lia Florey, Cameron Taylor, Rebecca Winter, Ana Claudia Franca-Koh, Samantha Herrera, Yazoume Ye

ICF International, Rockville, MD, United States

Sustained resources since 2000 have supported considerable gains in malaria control strategies, including insecticide-treated nets (ITNs) and intermittent preventive treatment in pregnancy (IPTp). Malawi has been a pioneer in adopting effective interventions, offering subsidized ITNs nationally by 2003 and encouraging IPTp by 1993. These programs contributed to a substantial increase in household ownership of at least one ITN from 27.4% in 2004 to 55.0% in 2012. Use of ITNs also increased during this time period among the traditional target groups of children under five years of age (2.8% in 2000 to 56.0% in 2012) and pregnant women (2.6% in 2000 to 50.7% in 2012). While utilization of antenatal services is high, IPTp coverage remains low, with only 53.8% of women receiving two or more doses in 2012. To assess equity of these interventions, Lorenz concentration curves and corresponding concentration index (C-Index) values were derived from 2000, 2004, and 2010 Demographic and Health Surveys and a 2012 Malaria Indicator Survey. Values approaching perfect equity across wealth quintiles (C-Index=0) show that equity has improved for all interventions studied. Increasing ITN ownership corresponded to improved equity over time (C-Index=0.29 in 2004 to C-Index=0.06 in 2012). Even larger gains were seen in ITN use by children under five (C-Index=0.47 in 2000 to C-Index=0.03 in 2012) and pregnant women (C-Index=0.45 in 2000 to C-Index=-0.01 in 2012). Equity in IPTp was stronger from an earlier date (C-Index=0.03 in 2000) and remained similar through 2012 (C-Index=0.06), suggesting that antenatal services are accessible and used equally across all wealth quintiles. The differences between ITN ownership and use and IPTp uptake show how equity may differ for various malaria control interventions. In order to achieve greater returns as Malawi moves toward universal coverage of all interventions and malaria transmission decreases, it will be important to acknowledge equity and focus resources on economic groups with outstanding needs.

1290

EXPLORATORY GEOSPATIAL MODELLING OF ENVIRONMENTAL FACTORS CORRELATED WITH PODOCONIOSIS IN ETHIOPIA

Yordanos B. Molla[1], Nicola Wardrop[2], JeJennifer Le Blond[1], Peter Baxter[3], Melanie J. Newport[1], Peter M. Atkinson[2], Gail Davey[1]

[1]Brighton and Sussex Medical School, Brighton, United Kingdom, [2]Geography and Environment, University of Southampton, Southampton, United Kingdom, [3]Institute of Public Health, University of Cambridge, Cambridge, United Kingdom

Ecological studies have linked podoconiosis (endemic, non-filarial elephantiasis) to red clay soils of volcanic origin, but the precise trigger of the disease is unknown. Histopathology investigations have demonstrated phyllosilicates, aluminium, magnesium and iron in the lower limb lymph node macrophages of both patients and non-patients living barefoot on these clays. We studied local spatial variation in disease prevalence and environmental factors with the aim of increasing understanding of disease pathogenesis. Fieldwork was conducted from June 2011 to February 2013 in 12 kebeles (administrative units) in northern Ethiopia. Geo-located prevalence data and soil samples were collected and analysed along with secondary geological, topographic, meteorological and elevation data. Soil data were analysed for chemical composition, mineralogy and particle size; and interpolated using regression kriging. Exploratory, univariate and multivariate regression analysis of podoconiosis prevalence in relation to primary (soil) and secondary (elevation, precipitation and geology) covariates was conducted. Following appropriate transformation to predict soil covariates, exploratory analysis indicated that podoconiosis prevalence was associated with clay minerals (smectite, kaolinite and mica), quartz (crystalline silica), iron oxide, and zirconium. The final multivariate model included smectite (OR = 2.76, 95% CI: 1.35, 5.73; p = 0.007), quartz (OR = 1.16, 95% CI: 1.06, 1.26; p = 0.001) and mica (OR = 1.09, 95% CI: 1.05, 1.13; p < 0.001). The association between podoconiosis prevalence and smectite, quartz and mica content of the soil suggests that further environmental, biomedical and toxicology studies on podoconiosis should focus on these soil characteristics.

1291

IS EARLY COMPLEMENTARY FOOD INTRODUCTION RELATED TO LOW INFANT WEIGHT-FOR-HEIGHT?

John D. McLennan

University of Calgary, Calgary, AB, Canada

Early complementary food introduction, by definition, undermines exclusive breastfeeding, as well as increases health risks for infants. A

broad array of liquids and solids are reported as introduced early in the diets of infants in many countries despite the promotion of exclusive breastfeeding for the first six months of life as optimal. Despite this pattern, there has been comparably little investigation into factors that may underlie early complementary feeding. Mothers' interpretation that their children's poor growth is a function of inadequacy of their breast-milk alone may be one factor, particularly in low resource settings of low- and middle-income countries. This study investigated whether more extensive early complementary feeding is related to lower child weight-for-height using data from the 2007 Demographic and Health Survey of the Dominican Republic. Of the 763 children under six-months of age with complete complementary feeding data, only 10.7% were classified as exclusive breast-feeders, although 79.6% were breastfeeding at the time of the survey. Among breast feeders who were non-exclusive, plain water and other milk types were the most common complementary products used. Baby cereal and items from the bread/noodles/grain group were the most common foods consumed. A summation of responses to 22 complementary food/liquid items consumed the day prior to the survey was used to index the extent of complementary food use. Inconsistent with the study hypothesis, this measure was not related to the Z scores of the children's weigh- for-height in either bivariate analysis or a multivariate model controlling for child age. Study findings are limited given the cross-sectional nature of the dataset and lack of variables on maternal perception of thinness, the latter which may provide a better index of maternal concern about child growth than the direct anthropometric measure used in this study. Nevertheless, other variables should be examined to identify key factors driving early complementary food introduction.

1292

QUALITY OF EMERGENCY NEWBORN CARE IN RURAL BANGLADESH, 2013

Wit Wichaidit[1], Mahbub Ul Alam[2], Davidson H. Hamer[3], Amal K. Halder[2], Leanne Unicomb[2], Pavani K. Ram[1]

[1]University at Buffalo, Buffalo, NY, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [3]Boston University, Boston, MA, United States

The neonatal mortality rate in Bangladesh is 26 deaths per 1000 live births; 14% of births are preterm. Signal functions, a list of critical interventions, were recently developed for emergency newborn care (EmNC) but their availability at national level has never been assessed in any country. Our objective was to assess the availability of EmNC signal functions at hospitals predominantly serving rural populations of Bangladesh. Directors of hospitals providing inpatient care to 50 villages, selected to be representative of rural Bangladesh, were interviewed about hospital infrastructure, staffing, and provision of four EmNC signal functions within the past 3 months (neonatal resuscitation, administration of antibiotics, administration of corticosteroids and provision of kangaroo mother care [KMC] for preterm births). Hospitals providing all 4 signal functions, with ≥3 staff on call (24-hour coverage), and an ambulance and phone for referring patients, were considered to provide high quality EmNC. Hospitals with ≥3 signal functions, ≥2 staff (or no 24-hour coverage), and a phone but no ambulance were considered to provide moderate quality EmNC. Hospitals with ≥2 signal functions and ≥1 staff and a phone provided low quality EmNC; the rest were considered sub-standard. Directors of 432 hospitals in 46 of 64 districts in Bangladesh took part; 383 hospitals (89%) were located in urban areas. Newborn care was available in 98% of hospitals. The most commonly available signal function was neonatal resuscitation (90%), followed by administration of antibiotics (86%) and corticosteroids (40%), and KMC (only 8% of facilities). KMC was more common in public hospitals (16% vs. 5%, P = 0.005). Quality of EmNC care was high in 11(3%) hospitals, moderate in 30%, low in 45%, and sub-standard in 22%. Inadequate availability of KMC and corticosteroids represent substantial barriers to providing high quality of care for particularly vulnerable preterm neonates. Efforts to motivate delivery at health facilities should be matched by strengthening EmNC at those facilities.

1293

INTEGRATED SURVEILLANCE FOR DISEASE CONTROL: A NEW ERA IN GLOBAL HEALTH?

Kate Zinszer[1], Sonia Menon[1], Lauren Caroll[2], Neil Abernethy[2], David Buckeridge[1]

[1]McGill University, Montreal, QC, Canada, [2]University of Washington, Department of Biomedical Informatics and Medical Education, Seattle, WA, United States

Donors, ministries of health, and other global health partners share the responsibility of ensuring the availability of valid and timely health indicators. There is growing recognition for the critical role these indicators have in the strategic development of effective responses to global health issues. Unfortunately, data fragmentation across diseases, countries, funding sources, and a wide range of clinical and laboratory data sources pose a barrier to timely estimation of accurate health metrics. Such fragmentation frustrates efforts to merge, analyze, and interpret data across multiple geographical scales, and hinders attempts to use these data to develop and share transparent, scalable tools for decision-making. The purpose of this project is to develop and evaluate 'proof-of-concept' tools and technologies to support the integration and use of global health data collected across a range of diverse sources. We intend to demonstrate the value of these tools and technologies for improving decision-making related to malaria control in Uganda and The Gambia. The first phase of our work entails developing a catalog of data sources for malaria control and describing how malaria control programs, funding agencies, and partners analyze and use these data to make programmatic decisions. The second and third parts of the project involve developing and evaluating the technology and software tools that will interact with the data sources to calculate and analyze valuable health metrics. At the completion of our project, which is anticipated to be November 2014, we will have developed an open-access prototype system that will support sharing of comparable data within and across countries. The system will include tools for supporting the effective use of data, and it will provide a mechanism for facilitating convergence towards common data standards to support the control of malaria and other priority diseases.

1294

MONITORING THE QUALITY OF ANTIMALARIAL DRUGS IN SENEGAL: A STAKEHOLDER PERSPECTIVE

Mirza Lalani

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

Poor quality antimalarials may have a deleterious effect on public health in malaria endemic countries. A national drug quality surveillance system may minimise the risk of such drugs appearing in the public and private sector, but little is known about these systems in developing countries. The aim of this study was to explore the perceptions of stakeholders on their institutional roles and responsibilities for assuring the quality of drugs, and strengths and weaknesses of the drug quality surveillance system in Senegal. In-depth qualitative interviews were conducted with 27 key stakeholders including representatives of the surveillance system authorities and treatment providers in the regulated public and private health sectors. Interviews focussed on two aspects of drug quality surveillance: 1) understanding the system context including its background, challenges faced and institutional roles and responsibilities of national authorities, and 2) identifying vulnerable components of the system that, if compromised, may increase the risk of poor quality antimalarials in Senegal. A health systems viewpoint was applied, allowing for inductive expansion of emergent themes in relation to the six building blocks of health systems. Preliminary analysis indicates that all stakeholders perceived the system to operate effectively and they had confidence in

the quality of antimalarials available in Senegal. Nonetheless, coordination amongst the different national authorities involved in assuring and monitoring drug quality, and between authorities and treatment providers, was recognised as a challenge. Differences in perceived quality and efficacy of antimalarials were often assumed to be related to their cost and country of manufacture. Insufficient drug storage conditions and the existence of an informal drug sector were seen as the two main risk factors for poor quality antimalarials in Senegal.Stringent drug regulation and a secure drug supply chain were perceived to contribute to the confidence in the quality of antimalarials available Senegal. However a lack of funding, issues of governance, inadequate human resources and an absence of monitoring of the informal sector (due to concerns that acknowledgment would legitimise its existence) all threaten to impair the progress that has been made by national authorities and external partners in assuring drug quality in Senegal.

1295

PERCEPTIONS OF HEALTH AND VULNERABILITIES ALONG THE INTER-OCEANIC HIGHWAY IN MADRE DE DIOS, PERU: RESULTS FROM QUALITATIVE RESEARCH

Valerie A. Paz Soldan[1], Valerie Baquerizo[2], Lara Schwarz[3], Gabriela Salmon-Mulanovich[4], Stella M. Hartinger[2], Daniel G. Bausch[1]

[1]Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States, [2]Universidad Peruana Cayetano Heredia, Lima, Peru, [3]McGill University, Montreal, QC, Canada, [4]U.S. Naval Medical Research Unit - 6, Lima, Peru

The Madre de Dios Region in the Peruvian Amazon is a biodiversity hotspot that has been highly affected by human settlement and land-use change since the construction of the inter-oceanic highway (IOH) that crosses the region. We explored societal impacts, quality of life issues, and vulnerabilities associated to the IOH, as part of a larger study focusing on transmission of rodent-borne diseases. Twelve focus group discussions with 83 community members and 21 key informant interviews with local leaders and health personnel were conducted in February 2014 in 8 communities along the IOH. Although people believe the IOH brought positive changes to their communities, they had an overall negative perception of the IOH. They attributed the increase in road accidents, crime and alcohol/drug consumption in recent years to the increase in human migration brought by the IOH. Lack of electricity and clean drinking water were common concerns. Findings suggest migrants tended to settle in the community outskirts, closer to intact rainforest, placing them at higher risk for emerging infectious diseases. For some jobs, people worked for long periods of time in remote locations in the rainforest, with its inherent risks. Several communities mentioned anemia as a significant health problem, but most communities now considered themselves healthy - dengue fever, leishmaniasis, malaria and gastrointestinal parasites were all cited as past problems. People recognized various types of rodents, and some complained about their role as pests, but did not express particular concern about diseases these may transmit. There are distinct differences between the communities north and southwest of the capital city Puerto Maldonado. While the main economic activities in all communities are logging, agriculture and Brazil nut collection, the southwest communities are often surrounded by illegal mining camps. As a result, they have much more support than northern communities from governmental and non-governmental organizations promoting diverse projects to improve the situation. Determining local peoples' perceptions of key issues and vulnerabilities in relation to health and the IOH will enable a greater understanding of how to approach current and future public health problems occurring in this region.

1296

INNOVATION TO ADDRESS DIAGNOSTIC NEEDS FOR RURAL POPULATIONS: INSIGHTS OF JUNIOR MEDICAL DOCTORS AT THE FRONTLINES OF RURAL CARE IN PERU

Cynthia F. Anticona Huaynate[1], Valerie A. Paz Soldan[1], Monica J. Pajuelo[1], Malena Correa[1], Holger Mayta[1], Richard A. Oberhelman[1], Mirko Zimic[2], Robert H. Gilman[3], Laura L. Murphy[1]

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

Diagnosis of common diseases can require specific technologies and depends on a skilled workforce, which are common limitations in rural settings. Few studies have assessed health providers' perceived needs for diagnostic tools and barriers they face in diagnosis - information that can guide innovation development and implementation. The aims of this study were to examine, from the perspective of medical doctors (MDs) in rural Peru, the needs and barriers associated with the diagnostic process, and solicit ideas for innovative solutions. Three focus group discussions (12 participants) and 18 individual semi-structured interviews were conducted with recent MD graduates who had completed their medical service in rural areas of the Highlands and Amazon basin in Peru in the last two years. Data were analyzed manually to explore trends in the main themes. The main diagnostic needs for infectious diseases included point of care (POC) tests for: i) the differential diagnosis of malaria vs pneumonia, ii) dengue vs leptospirosis, iii) tuberculosis, iv) vaginal infections and cervical cancer. Ultrasound was a perceived need for obstetric and intra-abdominal conditions. In specific locations, diagnostic tools for neurocysticercosis and for heavy metals toxicity were needed. Barriers impeding the diagnostic process included: distance to and high cost of referral facilities; cultural and linguistic issues; inefficient referral and laboratory systems; and inadequate telecommunication. Innovative ideas proposed by participants included: POC equipment such as a "rural ultrasound" and telemedicine services. Our findings show there is a high demand for improved diagnostic testing in rural communities, so a system based fundamentally on referrals is inadequate: rural doctors need more tools that are technologically and socially viable in context. National strategies supporting the development and implementation of diagnostic innovations are crucial for improving health services. This process should be informed by the perspectives of health providers in the underserved areas.

1297

AN ESTIMATION OF THE ECONOMIC BURDEN OF TYPHOID FEVER ILLNESS IN LOW AND MIDDLE-INCOME COUNTRIES

Vittal Mogasale[1], Brian Maskery[1], R. Leon Ochiai[2], Jung Seok Lee[1], Thomas F. Wierzba[1]

[1]International Vaccine Institute, Seoul, Republic of Korea, [2]International Vaccine Institute, Seoul, Republic of Korea

Background Estimation of typhoid cost of illness in low and middle income countries is important in cost-effectiveness analysis and priority setting for typhoid prevention and control activities such as vaccine introduction. In this paper, we present an estimate of total costs and cost per episode of typhoid fever illness in low and middle income countries by United Nations sub-regions. Methods A decision tree model was developed to represent the clinical outcomes of typhoid illness. The percentage of typhoid cases corresponding to each arm were estimated from a literature review. The indirect costs were measured based on a five country typhoid cost of illness study. The costs related to laboratory diagnosis, service delivery, and medicines were assessed based on literature and World Health Organization data base. Direct and indirect costs were estimated in 2010 United States dollars ($), and segregated by outpatient and inpatient status. Findings The estimated total annual typhoid fever cost of illness in low and middle income countries was $519 million (95% CI=300 million to $836 million) of which 46% came from South Asia. The average cost of typhoid illness per episode was $44 (95% CI= $25 to $69), which

comprised 28% direct costs and 78% of indirect costs. Average cost per outpatient was $31(95% CI=$16 to $52) while the cost per inpatient was about $191(95% CI=129 to 276). The predominant cost drivers were indirect costs, number of episodes in the region and hospitalization rates. Interpretation The main challenge was obtaining the probabilities of health events for the decision tree model due to a lack of data. Our cost estimates were conservative, as typhoid relapse, typhoid death, development of long-term carrier state, and gall bladder cancer were not included. South Asia and East Africa should be the priority for typhoid prevention and control activities due to their high economic burden. There is a need for collecting improved and geographical representative epidemiological and cost data for typhoid fever.

1298

CURRENT EFFORTS OF PHARMACEUTICAL COMPANIES TO ADDRESS THE NEED FOR PEDIATRIC PRODUCT DEVELOPMENT: AN ANALYSIS OF THE R&D PIPELINE

Delphi G. Coppens, Anna V. Massey, Jayasree K. Iyer

Access to Medicine Foundation, Haarlem, Netherlands

The majority of medicines have primarily been developed for use by adults. Treating children with these medicines can be problematic. Children of different ages and weights need different dosages and dosing forms (such as oral liquids) to ensure efficacy, safety, and compliance. Certain age groups also metabolise medicines differently, which needs careful evaluation. In 2007, the World Health Organization (WHO) published its first Essential Medicines List for Children (EMLc). It revealed the need for more evidence on the efficacy and safety of many essential medicines for use in children (including systemic reviews and product development). The WHO repeated this call in 2013 in the update of its report 'Priority Medicines for Europe and the World'. To address such unmet needs, pharmaceutical companies can play a key role, as they have deep expertise on formulation and manufacturing, as well as intellectual property rights on adult medicines. To assess their response, we present a unique analysis of the paediatric R&D activities currently being undertaken by 21 research-based pharmaceutical companies with the highest global market capitalization (as measured by the 2014 Access to Medicine Index). The Index measures the extent to which these companies address the issue of access to medicine for 47 high-burden diseases, with significant overlap of the EMLc. As such, this analysis maps the priority paediatric R&D needs that these companies are addressing, and where gaps remain. Preliminary results show that more than 50% of these companies are involved in paediatric R&D for EMLc diseases, including developing adapted formulations and vaccines specifically for children. The vast majority are medicines target communicable diseases, including tuberculosis and malaria. There are also examples of adapted formulations for non-infectious diseases, including diabetes mellitus. Although limited, adapted formulations for neglected tropical diseases and neonatal health indicates progress in these disease areas as well. This study will be completed in September 2014.

1299

THE PROCESS OF CLINICAL TRIAL IMPLEMENTATION: PARTNERSHIP BETWEEN THE TRIAL RESEARCH TEAM AND LOCAL HEALTH STAFF IN BURKINA FASO, GHANA AND ZAMBIA

Léa Paré Toé[1], Frank Baiden[2], Sebastian Hachizovu[3], James Akizili[2], Halidou Tinto[1], Shepherd Khondowe[3], Yacouba Cissao[1], Raffaella M. Ravinetto[4], Umberto D'Alessandro[5], Koen Peeters Grietens[6], Maxime K. Drabo[1]

[1]Institut de Recherche en Sciences de la Santé, Bobo Dioulasso, Burkina Faso, [2]Navrongo Health Research Center, Navrongo, Ghana, [3]Tropical Diseases Research Centre, N'dola, Zambia, [4]Department of Clinical Sciences, ITM, Antwerp, Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Antwerp, Belgium, [5]Department of Clinical Sciences, ITM, Antwerp, Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Medical Research Council, Fajara, The Gambia, Antwerp, Belgium, [6]Department of Clinical Sciences, ITM, Antwerp, Belgium

Clinical trials are essential for health research, particularly for the assessment of new health interventions. In order to ensure patients' protection and research quality, these trials follow strict procedures that may not be available for routine practice in poor settings. We researched the partnership between trial teams and local health providers, more specifically whether the technical knowledge gained from the trial is beneficial for local health services in trial settings in Burkina Faso, Zambia and Ghana. We used a quantitative survey and qualitative research methods to collect data from professionals of the Ministries of Health, local health providers and clinical researchers in settings in the respective countries where trials were on-going or had recently ended and compared them to control sites where no trial research had been done. Local health services benefit from the presence of research teams but there is room for improvement. Improved communication with local health staff and their regular involvement in training opportunities provided to the research team, among other factors, could bring long-term improvements to quality of care also in local routine health care, which would be a positive added value of the research in local settings. Clinical trials are beneficial for resource-poor communities not only because of their primary objective of investigating better treatments, but also for the improved quality of care provided during the trial. Nevertheless, local health services could additionally benefit from the trial implementation process, extend its positive impact on routine health care provided to the local populations.

1300

HOSPITALIZATIONS AND DEATHS DUE TO DIARRHEAL AND RESPIRATORY DISEASES AMONG CHILDREN UNDER FIVE YEARS OF AGE-HAITI, 2011-2013

Kavita Vinekar[1], Mentor Ali Ber Lucien[2], Nicholas Schaad[3], Eyal Leshem[3], Ikwo Oboho[3], Gerard Joseph[2], Stanley Juin[4], Fatimah Dawood[3], Umesh Parashar[3], **Rania A. Tohme**[3]

[1]University of Michigan, Ann Arbor, MI, United States, [2]Ministry of Public Health and Population, Port-au-Prince, Haiti, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States, [4]Centers for Disease Control and Prevention, Port-au-Prince, Haiti

Diarrheal and respiratory diseases are leading causes of morbidity and mortality among children aged <5 years in developing countries; however, data on the burden of these diseases in Haiti are scarce. We conducted a retrospective review of hospital admission registries for children aged <5 years during January 2011 through December 2013 in six major hospitals in Haiti. We recorded numbers of all-cause, diarrheal and respiratory disease admissions by age group, hospital, and epidemiologic week. Diarrheal diseases included diarrhea, gastroenteritis, dehydration, parasitosis, cholera, amoebiasis, dysentery, shigellosis, giardiasis, food poisoning and hypovolemic shock. Respiratory diseases included bronchitis; bronchioloitis; acute sinusitis, epiglottitis, tracheitis, viral rhinitis,

pharyngitis or respiratory illness; bronchopneumonia, asthma, influenza, influenza-like symptoms, bronchiectasis, pneumonitis, laryngotracheitis, croup, pleural effusion, respiratory failure/distress, empyema, pleurisy, apnea, shortness of breath, tachypnea, wheezing, stridor, cough, diphtheria. A total of 31,565 hospital admissions and 1763 deaths were recorded among children aged < 5 years at the six sites. Diarrheal diseases accounted for 8063 (26%) hospitalizations and 224 (13%) deaths. Diarrheal diseases accounted for 39%, and 36% of hospitalizations in children aged 6-11 months and 12-23 months, respectively. While children aged 0-5 months constituted 25% of all diarrheal disease hospitalizations, diarrheal diseases accounted for only 15% of all hospitalizations in this age group. Diarrheal disease admissions peaked in January-April before the rainy season. Respiratory diseases accounted for 9183 (29%) hospitalizations and 301 (17%) deaths. Children aged 0-23 months accounted for 76% of all respiratory disease admissions. Children aged 0-5 months had the lowest proportion of hospitalization due to respiratory diseases (19%) while children aged 6-23 months had the highest (38%). Respiratory disease hospitalizations followed a bimodal seasonal pattern, with peaks during May-June and October-December. Diarrheal and respiratory diseases constitute a significant health burden among children aged < 5 years in Haiti. Having these data before rotavirus and pneumococcal vaccine introduction will be important to monitor the impact of vaccines and other health interventions.

1301

MENTORSHIP FOR GLOBAL HEALTH RESEARCHERS

Hazel E. McCullough[1], Donald C. Cole[2], The Canadian Coalition of Global Health Research International Mentorship Working Group[3]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Dalla Lana School of Public Health, Toronto, ON, Canada, [3]University of Toronto, Toronto, ON, Canada

Mentorship plays a critical role in the development of global health researchers and the strengthening of health research capacity in both high income countries (HIC) and low and middle income countries (LMIC). In 2012 the Canadian Coalition of Global Health Research (CCGHR) brought together colleagues from Canada, Argentina, Chile, Guatemala, Kenya, and the UK/Europe to collate current examples of mentorship practice in global health research (GHR). Using a research story and narrative approach, a set of eleven GHR mentorship research stories were developed on diverse mentorship experiences and programs. Teleconferences and an online project management platform were used to manage global communication, to guide the story development through peer review and reflection, and to promote conjoint analysis. A tabular and curatorial analytic approach highlighted the unique aspects of each of the stories but also provided insights into the challenges, benefits and commonalities that arise in GHR mentorship. The fundamental principles of mentoring were used to develop diverse programs to meet the needs and contexts of the global health researchers for which they were developed. These included mentorship programs for researchers in the field of: global mental health and substance use in Kenya, tobacco control in Argentina, malaria in Sub-Saharan Africa, chronic disease in Guatemala, Chilean led research training in Africa, and Canadian led interdisciplinary programs to create environments for mentorship, bring together mentors and mentees in GHR from HICs and LIMCs in globally hosted summer institutes, and build communities of practice at three Canadian universities. Key outcomes from the project include online access to the mentorship stories in English, French and Spanish, and the creation of an international working group of global health researchers engaged in GHR mentorship within HICs and LIMCs; and who are well situated to contribute to the emergent literature in GHR mentorship.

1302

APPLICATION OF THE UNIFIED THEORY OF ACCEPTANCE AND USE ON COMMUNITY HEALTH VOLUNTEERS' ACCEPTANCE OF MHEALTH

Baaba da-Costa Vroom[1], Richmond Aryeetey[1], Richard Boateng[2], Francis Anto[1], John Gyapong[1]

[1]School of Public Health, University of Ghana, Legon, Accra, Ghana, [2]University of Ghana Business School, Legon, Accra, Ghana

Studies have been conducted to examine the potential benefits of the use of mobile health (mHealth) tools to improve the work of community health volunteers (CHVs). The use of mHealth is expected to enable CHVs take on more challenging tasks and enable them produce more timely and accurate data for their health sectors. However, little is known about CHVs level of readiness and acceptance to use mHealth. This study therefore aims to determine the factors that influence CHVs acceptance and intention to use mHealth applications to collect and report data during mass drug administration (MDA) for lymphatic filariasis control. All CHVs in two districts in Ghana (approx. 300) will complete questionnaires to determine their readiness to adopt and use mHealth for lymphatic filariasis treatment coverage data reporting. The Unified Theory of Acceptance and Use of Technology (UTAUT) model has been used to determine the probability of acceptance of new technology among specific groups to whom new technology tools are being introduced and who are potentially less inclined to accept them. Though this tool has been used to validate technology acceptance among formal health sector workers, little is known about technology acceptance by CHVs. CHVs acceptance and intention to use of mHealth will be measured by four constructs; performance expectation, effort expectation, social influences and other facilitating conditions. The UTAUT model will provide a means to assess the probability of mHealth acceptance and intention to use technology among CHVs. It will also provide theoretical and practical implications for sustaining health sector adoption of mHealth in a low resource setting. This study will be completed by June 2014.

1303

ENVIRONMENTAL PUBLIC HEALTH IMPACTS OF DUST AND SAND STORMS IN CENTRAL AND SOUTHWESTERN REGIONS OF IRAQ

Mohammed A. Kalkhan[1], Sounak Chakraborty[2], Keshab Gangopadhyay[3]

[1]Center for Remote Sensing, University of Kufa, Kufa, Iraq, [2]Department of Statistics, University of Missouri-Columbia, Columbia, MO, United States, [3]Department of Electrical and Computer Engineering, University of Missouri-Columbia, Columbia, MO, United States

Recent severe drought and water shortages in Iraq have led to an increased incidence of devastating dust and sand storms. Drought, degradation of the environment, conflict and climate change are imposing a significant impact on public health with a spatial dimension. Our geospatial approach includes modeling the complex determinants of dust and sand storm development and propagation using remote sensing satellite imagery, geospatial information systems, and geostatistical analysis using modeling techniques with multiscale nested sampling designs. Predictions from this approach are relevant to many environmentally dependent living ecosystems (human, agricultural, water) and can be linked to socio-economic and public health consequences. We account for site specific land ecosystem conditions in Iraq by adapting a geospatial thematic mapping technique that has been successfully used in other types of semi-arid environments at a broad (i.e., "landscape") scale. We use these dynamic geospatial modeling and thematic mapping techniques as a predictive tool to improve the forecasting of dust and sand storms throughout Iraq to better understand the relationship between environment and ecosystem degradation and its impact on public health. A significant outcome of this research is geared towards the analysis of dust and sand dynamics and their effects on sustainable land use decision

making that is important for environmental public health. These geospatial models can inform communities, regional land managers, government policymakers, other constituents and diverse stakeholders regarding the potential impacts of increased dust and sand storms on public health in Iraq.

1304

PRELIMINARY RESULTS OF SENTINEL SURVEILLANCE OF UNDIFFERENTIATED FEBRILE ILLNESSES IN GEORGIA IN 2013

Tinatin Kuchuloria[1], Manana Makhviladze[2], Marine Kanashvili[2], Teona Mikautadze[2], Nora Kokaia[3], Manana Makharadze[3], Nana Gongadze[4], Marina Endeladze[5], Lana Gatserelia[5], Marine Karchava[5], Tengiz Tsertsvadze[5], Maiko Chokheli[6], Nana Mamuchishvili[6], Lile Malania[6], Nino Kiknadze[6], Nazibrola Chitadze[6], Marina Chubinidze[6], Tamar Chikviladze[6], Paata Imnadze[6], Nino Trapaidze[1], Robert G. Rivard[7], Helen Freyberger[8], Mikeljon P. Nikolich[8], Christian T. Bautista[8]

[1]United States Army Medical Research Unit-G/Walter Reed Project Georgia, Tbilisi, Georgia, [2]V. Bochorishvili Sepsis Center, Tbilisi, Georgia, [3]Institute of Medical Parasitology and Tropical Medicine, Tbilisi, Georgia, [4]Medical City Hospital, Kutaisi, Georgia, [5]Infectious Diseases, AIDS and Clinical Immunology Research Center, Tbilisi, Georgia, [6]National Center for Disease Control and Public Health, Tbilisi, Georgia, [7]United States Army Medical Research Institute for Infectious Diseases, Fort Detrick, MD, United States, [8]Walter Reed Army Institute of Research, Silver Spring, MD, United States

This surveillance project seeks to determine the burden of infectious agents of undifferentiated febrile illnesses (UFI) and hemorrhagic fever syndrome (HFS). From June to December of 2013, patients≥4 years of age with a temperature of ≥38°C for ≥48 hours or HFS were enrolled. In addition to blood culture, serologic testing (ELISA) was conducted to detect antibodies against *Leptospira* spp., *Brucella* spp., *Coxiellaburnetii*, CCHF virus, hantavirus, Spotted Fever Group (SFGR), Scrub Typhus group (STG), and Typhus group (TG) *Rickettsiae.* Hantavirus ELISA results were confirmed by IgM/IgG IFA.There were 245 patients enrolled in the study; 30 (12%) returned for the voluntary follow-up visit. Blood culture was positive for only 7 (2.8%). Fourteen (5.7%) patients tested positive by both IgM and IgG against *Brucella* spp. and 29 (11.8%) demonstrated only IgG positivity. *Brucella melitensis* was isolated from one patient. Additionally, *Leptospira* spp. IgM, SFG IgG and *C.burnetii* IgM was positive in 23 (9%), 9 (3.6%) and 7 (2.8%) patients, respectively. Of patients positive for hantavirus, 17 (6.9%) were positive for IgM and 7 (2.8%) were positive for IgG using ELISA. Six of the IgM and 4 of the IgG hantavirus positive samples have been retested using IgM/IgG IFA and were negative. Three (1.2%) patients demonstrated both IgM/IgG and 8 (3%) only IgG positivity against CCHF virus, but none of them had a recent or present history of HFS.These initial results suggest that brucellosis is one of the leading causes of the UFI in Georgia. Additionally our findings suggest that leptospirosis, rickettsiosis and Q-fever are diseases requiring a high index of suspicion by physicians and improved laboratory capacity for correct diagnosis and treatment to take place. Initial ELISA findings on hantavirus and CCHF virus suggest that a more specific test is needed. Surveillance will continue until 2016 to improve the detection and treatment of selected diseases with an emphasis on developing capacity for diagnosis and laboratory confirmation.

1305

A PRELIMINARY ANALYSIS OF THE QUALITY OF PEDIATRIC MEDICINES SUPPLIED BY PRIVATE WHOLESALERS IN KINSHASA, DRC

Benedetta Schiavetti[1], Daniel Ngeleka Mutolo[2], Roland D. Marini[3], Bart De Spiegeleere[4], Epco Hasker[1], Josiane Van Der Elst[5]

[1]Institut of Tropical Medicine Antwerp, Antwerp, Belgium, [2]Direction de la Pharmacie et du Medicament, Kinshasa, Democratic Republic of the Congo, [3]University of Liege, Liège, Belgium, [4]University of Ghent, Ghent, Belgium, [5]Agence Fédérale du Médicament et Produits de Santé, Bruxelles, Belgium

The global pharmaceutical market is characterised by multiple qualitative standards. Low and middle-income countries are particularly permeable to poor quality products: the proportion of substandard medicines in sub-Saharan Africa ranges from 12% to 48%, though accurate figures are not available, especially for paediatric medicines. In the Democratic Republic of Congo, one of the prime objective of the national Health Development Plan 2011-2015 is the reduction of infant mortality and a transversal objective is to ensure that 80% of the medicines available is of good quality. In 2013, the introduction of Minilabs® revealed the presence of substandard products but the actual prevalence of poor-quality medicines in the country is unknown. In the context of a North-South bilateral cooperation program, a cross-sectional survey on the quality of products available in the private market in Kinshasa was carried out with the national medicine regulatory authority (DPM). Paediatric formulations of amoxicillin, artemether/lumefantrine and paracetamol were selected as tracers of medicine quality, based on 8 public health criteria and on the results of informal interviews. Covert shoppers purchased a defined quantity of packs of each brand available in all the licensed wholesalers of the city. To obtain a representative subsample of the most marketed products, the inspectors of the DPM collected the yearly distribution figures from the wholesalers. From all the purchased samples, a weighted subset of 100 for each molecule was randomly selected for analysis. The DPM performed the visual inspection on all the purchased products while the subsample was sent in Belgium and tested according to the United States Pharmacopoeia (USP) analyses. The Medicine Quality Assessment Reporting Guideline was followed for reporting and the information arising from visual inspection was used for identifying lacks in the current legislation. Between 7th and 16th April 2014, 417 samples were collected: 86 paracetamol tablets, 143 amoxicillin and 188 artemether/lumefantrine, both powders for suspension. The visual inspection will be performed in May and pharmacopoeial analyses in August 2014. The overall results are expected by October 2014 and will be presented.

1306

GLOBAL IMMUNIZATION POLICY FORMATION FOR NEW VACCINES

Olwen Jaffe[1], M. Imran Khan[1], Adwoa D. Bentsi-Enchill[2], Ciro de Quadros[1]

[1]Sabin Vaccine Institute, Washington, DC, United States, [2]World Health Organization, Geneva, Switzerland

Stakeholder involvement in the immunization policymaking process is complex and occurs at many different levels. Similarly, the process from vaccine development to implementation and use in an immunization program has many different phases and is typically very lengthy. To ensure that vaccines are having the maximum impact, there is need for vaccine developers to incorporate public health use considerations into these early phases. Implementing vaccine policies can often prove challenging for many countries, yet vaccine developers often overlook these policy challenges In this review paper, international immunization policy is understood to be the immunization policy set by the World Health Organization (WHO) for the purpose of informing regional and national immunization practices and regional immunization policy is the immunization policy set by the six WHO regional offices. To understand

the immunization policymaking process, a review of available documents outlining the various immunization policymaking sub-processes and WHO committees involved in the immunization policymaking process was conducted in concert with a review of available articles on the details of Strategic Advisory Group of Experts (SAGE) and SAGE's working groups, as well as other advisory bodies of the WHO that contribute to the fulfillment of SAGE's mission. Recommendations for immunization policy are made, beginning with at WHO and continuing through the WHO Regional Offices and their respective Immunization Technical Advisory Groups (ITAG), with most finishing with the National Immunization Technical Advisory Groups (NITAG), although others have state or municipal level immunization advisory groups that make even more specific recommendations. Though immunization requirements and laws can only be made at the national, state, or municipal level, the WHO and its Regional Offices play a major role in formulating and influencing national immunization policies. While there is uniformity in the process across national and regional borders, there are stark differences in the actual practice of formulating and/or adopting immunization policy across municipalities, or city or town governments, and countries. This paper attempts to clearly delineate the policymaking process for immunization recommendations from beginning to end, in addition to bringing simplicity and clarity to the intricate process for the average stakeholder.

1307

THE EFFECT OF MASS AZITHROMYCIN DISTRIBUTIONS ON CHILDHOOD MORTALITY: BELIEFS AND ESTIMATES OF EFFICACY

Kieran S. O'Brien, Craig W. See, Jeremy D. Keenan, Nicole E. Stoller, Sun N. Yu, Travis C. Porco, Thomas M. Lietman

F.I. Proctor Foundation, University of California San Francisco, CA, United States

A single cluster-randomized trial conducted in Ethiopia found that mass distribution of azithromycin reduced childhood mortality by 50% in the first year (relative rate, 0.50; 95% confidence interval, 0.29 - 0.86). The magnitude of the observed effect was surprising given that other effective population-level interventions have resulted in more modest benefits. To further investigate, the relative risk of childhood mortality in communities given mass azithromycin distributions was estimated using two different methods: an expert survey and a Bayesian analysis of the cluster-randomized trial. Experts in public health, infectious disease, and demography were asked to estimate the true effect of oral azithromycin distributions on childhood mortality. Separately, an empirical Bayesian estimation of the efficacy was performed. This estimation was determined given the randomized trial's results and prior estimates based on the efficacy of effective non-antibiotic population-level interventions, including vitamin A supplementation and chemoprophylaxis for malaria. The surveyed experts believed mass azithromycin lowers childhood mortality (relative risk, 0.83; 95% credible interval, 0.70 - 1.00). The relative risk from the Bayesian analysis was 0.71 (95% credible interval, 0.39 - 0.93). Both expert opinion and the Bayesian analysis suggest that azithromycin may have a true mortality benefit, but that the most likely effect is smaller than that found in the single available randomized controlled trial. Survey respondents may have used prior information about other beneficial population-level interventions to inform their opinions about the efficacy of mass azithromycin. A large multi-site randomized controlled trial will be necessary to confirm a mortality benefit from mass azithromycin treatments and assess the magnitude of any such benefit.

1308

RECOMMENDATIONS FOR VALID CONSENT FOR RESEARCH WITH ADOLESCENTS IN LOW-INCOME SETTINGS

Phaik Yeong Cheah[1], Michael Parker[2]

[1]*Mahidol Oxford Tropical Medicine Research Unit, Bangkok, Thailand,* [2]*University of Oxford, Oxford, United Kingdom*

Paediatric research is particularly relevant in the tropics where paediatric disease is such an important cause of morbidity and mortality. The current model for consent – where children provide assent (defined as "active agreement") for medical research and their parents must also consent – is not always appropriate, especially in low-income settings. We argue that assuming the research carries minimal risks and meets international ethical guidelines, more emphasis should be placed on the child's wishes. We propose that the default position should be that children who are able to provide valid consent should consent for themselves regardless of age. Many older children (adolescents) in low-income settings have adult responsibilities, may be parents themselves or may be estranged or living independently and not have parents or guardians to look after their interests. The requirements for a valid adolescent's consent should be the same as for adults: (1) the adolescent must be competent, and have the ability to reasonably understand and retain the information, weigh the options and make a decision; (2) the adolescent must be appropriately informed, meaning that the information must be presented in understandable language and illustrated by meaningful examples, and address concerns that are important to adolescents such as stigma and missing school; and (3) the consent must be voluntary and not coerced, taking into consideration that adolescents can easily take to praise and rewards, and may be afraid of adults and those in authority. Apart from these usual requirements, we propose two additional elements: (1) the adolescent must be genuinely mature, meaning he or she has had the life experiences necessary to make such decisions, is able to understand difficult concepts like research, altruism, participant responsibilities and the impact of participation on his or herself and others; and (2) the adolescent should be sufficiently independent: having his or her own accommodation, the ability to travel to attend follow-up visits, and a job or being a parent themselves.

1309

STATISTICAL UNCERTAINTY IMPOSES INHERENT LIMITS ON THE EFFICACY OF TARGETED DISEASE CONTROL

Alex Perkins

University of California Davis, Davis, CA, United States

Contributions of different individuals, groups, or geographic areas to the transmission of infectious diseases are often highly heterogeneous. One of the primary motivations for understanding transmission heterogeneity is the possibility of targeting control measures, such as vaccines, drugs, or insecticides, on individuals, on certain groups, or in areas that make greater contributions to transmission than others. Any effort to target controls must, however, be performed on the basis of some set of measurable factors presumed to be predictive of potential contributions to transmission. The success of efforts to target controls is determined then by the predictive capacity of the factors on which targeting is performed. In light of inherent limits on the capacity of any measurable factor to predict transmission potential, I extend a general and well-known mathematical framework to account for this type of uncertainty. For a given degree of transmission heterogeneity (e.g., 20% of individuals account for 80% of transmission, or the "20/80 rule"), I show how the proportion of variation in transmission potential explained by a set of predictive factors (i.e., R^2) determines the relative benefit from targeting in terms of reducing 1) the critical vaccination proportion, 2) the invasion probability of an emerging pathogen, and 3) the expected size of an outbreak. For the extent of transmission heterogeneity displayed in several well-studied disease outbreaks, I show that significant enhancement of the effectiveness of controls from targeting requires having factors to guide

targeting that explain a substantial proportion of variation in transmission potential. To conclude, I highlight diseases for which factors that could be used to guide targeting appear to be informative, as well as diseases for which predictive factors are unlikely to be found or for which the potential of such factors is not well known. These results highlight the importance of studying factors that underlie transmission heterogeneity and rigorously assessing their predictive capacity.

1310

REAPPRAISING END-OF-LIFE CARE IN THAILAND: A REVIEW OF POLICY AND PRACTICE COMPARED TO THE USA

Rapeephan R. Maude[1], Richard J. Maude[2]

[1]Department of Medicine, Saint Vincent Hospital, Worcester, MA, United States, [2]Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand

In Thailand it is not standard practice to ask patients and family members about code status (do not resuscitate, do not intubate or comfort-measures only) on admission to hospital or to allocate health care proxies. Many Thai patients have not considered these issues prior to the occurrence of critical illness. There are around 3 physicians per 10,000 people in Thailand compared to 24 in the USA, and few work in primary care. The cultural differences are not insurmountable and the US approach to terminal illness may be of benefit to patients in Thailand. A literature review was performed to compare and contrast current practice regarding cardiopulmonary resuscitation and end-of-life care in Thailand with that in the USA highlighting differences in knowledge, attitudes and cultural contexts. We propose that in Thailand early discussion of code status and appointment of a health care proxy should be adopted in hospitals to limit potential unnecessary discomfort and help provide appropriate care for patients with poor prognosis. This will require changes in health policy and training of healthcare providers and education of patients.

1311

MICRONUTRIENT SUPPLEMENTATION DURING PREGNANCY AND ANEMIA IN THE POST-PARTUM PERIOD AMONG WOMEN IN BOLIVIA'S ANDEAN PLATEAU

Adam Lipus[1], Kaitlyn Stanhope[1], Anna Fabiszewski de Aceituno[1], Paulina A. Rebolledo[1], Meghna Srinath[1], Rita Revollo[2], Volga Iñiguez[3], Parminder Suchdev[1], Juan S. Leon[1]

[1]Emory University, Atlanta, GA, United States, [2]The Population Council, La Paz, Plurinational State of Bolivia, [3]Universidad Mayor de San Andrés, La Paz, Plurinational State of Bolivia

Anemia contributes substantially to global morbidity in children and in women of reproductive age and can negatively influence maternal and neonatal outcomes if present during pregnancy. Micronutrient deficiencies (e.g., iron) underlie a substantial portion of the burden of anemia. Thus there is a need to quantify the prevalence of anemia as well as attitudes regarding and acceptability of micronutrient interventions among mothers and pregnant women. In Bolivia, the national universal health plan includes micronutrient supplementation for pregnant and post-partum women (pills containing iron, folic acid, and vitamin C) and for children age 6-24 months (multiple micronutrient powders). Our study assessed anemia status, access to and use of micronutrient supplements, and perceptions regarding the acceptability of supplementation among a predominantly indigenous population of mothers in El Alto, Bolivia, located in the Andean Plateau. Mothers (n=381) of one-month old infants recruited at well-child visits at two hospitals from May 2013 to March 2014 completed interviews on socio-demographic characteristics and prior use of micronutrient supplements. Researchers also performed Hemocue on venous samples and adjusted hemoglobin cutoff values for anemia according to altitude. Promisingly, 89% of mothers reported receiving iron pills during pregnancy, 76% reported taking iron, and only 24% were found to be anemic. However, more than a third of the women who took iron pills reported difficulty in taking these supplements. Similarly, less than a third of women reported having given their age-eligible children multiple micronutrient powder sachets, and only 47% of these women believed that other women would want to use them during pregnancy. These results suggest that coverage of multiple micronutrient supplementation in children lags behind that of iron supplementation of pregnant women. Furthermore, efforts to improve the desirability of these supplements may be necessary in order to maximize adherence among those who receive them.

1312

PREVALENCE OF EARLY-ONSET NEONATAL INFECTION AMONG NEWBORNS OF MOTHERS WITH BACTERIAL INFECTION: A SYSTEMATIC REVIEW AND META-ANALYSIS

Grace J. Chan[1], Abdullah H. Baqui[2], Anne C. Lee[3], Jingwen Tan[2], Robert E. Black[2]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [3]Brigham and Women's Hospital, Boston, MA, United States

Although neonatal infections cause a significant proportion of deaths in the first week of life, little is known about the burden of neonatal disease originating from maternal infection or colonization globally. We estimate the prevalence of vertical transmission - the burden of neonatal infection among newborns exposed to maternal infection.We searched Pubmed, Embase, Scopus, Web of Science, Cochrane Library, and WHO Regional Databases for studies of maternal infection, vertical transmission, and neonatal infection. Studies that measured prevalence or incidence of bacterial vertical transmission were included. 122 studies met the inclusion criteria. Random effects meta-analyses were used to pool data to calculate prevalence estimates of vertical transmission. The prevalence of early onset neonatal lab-confirmed infection among newborns of mothers with lab-confirmed infection was 17.2% (95%CI 6.5-27.9). The prevalence of neonatal lab-confirmed infection among newborns of colonized mothers was 1.1% (95%CI 0.2-2.0). The prevalence of neonatal surface colonization among newborns of colonized mothers ranged from 30.9-45.5%. The prevalence of neonatal lab-confirmed infection among newborns of mothers with risk factors ranged from 2.9-19.2%. Only seven studies (5.7%) were from high neonatal mortality settings. Considerable heterogeneity existed between studies given the various definitions of infection, colonization, and risk factors of infection. The prevalence of early-onset neonatal infection is high among newborns of mothers with infection or risk factors for infection. More high quality studies are needed particularly in high neonatal mortality settings to accurately estimate the prevalence of early-onset infection among newborns at risk.

1313

TRAINING LAYPERSONS BASIC TRAUMA TECHNIQUES IN LOW-INCOME COUNTRIES

Amina Merchant, Kelly Mcqueen

Vanderbilt University, Nashville, TN, United States

Trauma accounts for over 300 million years of healthy life and 11% of the disability-adjusted life years (DALYs) worldwide. Reduction of DALYs and mortality are linked to adequate prehospital care and decreased transport times to definitive care. Given the financial and resource constraints in low-income countries, simple but systematic prehospital training programs for laypersons have been implemented in rural villages to stabilize patients. Most prehospital deaths are the result of airway compromise, respiratory failure or uncontrolled hemorrhage; all three of these conditions can be addressed by laypersons using basic first aid measures. The hypothesis is that basic prehospital and primary hospital interventions made by layperson first responders and healthcare personnel will decrease mortality and increase the number of capable first responders. In order to test this hypothesis, communities with hospitals that advertise surgical capacity in Mozambique were assessed. Six hospitals and communities served as the intervention group that receives training on four basic resuscitative

and stabilizing efforts in their native language in the Zambesia province of Mozambique. Community members received a four-hour seminar that taught four basic resuscitative and stabilizing interventions prior to transport by ambulance or taxi/bus. These techniques include a modified ABCD (airway, breathing, circulation, disability) noted in developed nations. A is for airway opening that allows victims to receive oxygen by simply opening their mouths and removing any foreign objects if present. B is for bleeding – participants learned how to apply compression or a tourniquet. C represents cervical spine immobilization with simple tools. D is for disability which is reduced by transporting victims with a flat, immobile, safe method. Hospital personnel received the same ABCD training as the community with two additions – vital sign monitoring and IV fluid resuscitation as they are markers of shock and injury. Pre- and post- tests were administered to participants in their native language. Results of the study suggest community members can be trained in basic resuscitative techniques. In conclusion, while laypersons and hospital personnel may receive and feel comfortable administering basic resuscitation techniques, further data must be collected to see if this intervention improves mortality.

1314

PAPER TEST CARDS FOR SCREENING PHARMACEUTICAL QUALITY IN THE FIELD

Nicholas Myers, Abigail Weaver, **Marya Lieberman**

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

This presentation will describe a "lab on paper" that can characterize low quality pharmaceutical dosage forms and detect active pharmaceutical ingredients in falsified "herbal" medicines. The test card carries out a dozen color tests in parallel in under 5 minutes, producing a characteristic "fingerprint" of colors in the readout area. Pharmaceutical products which contain little or no active ingredient or which include substitute ingredients give different fingerprints from authentic products, as do "herbal" medicines that are actually spiked with pharmaceutical ingredients. The test cards can be read by eye or with an image analysis program. They are portable, easy to use, and testing of dosage forms can be done in minutes on the corner of a desk. In blinded lab validation studies, the sensitivity and selectivity values for detection of very low quality antibiotics, antimalarials, and tuberculosis medicines are measured as more than 90%. In this presentation, correlations between paper test card results by naive and expert readers and between test card results and HPLC analysis of field samples will be presented. Samples include authentic and falsified drugs as well as "herbal" medications provided by collaborators in Kenya, the US Food and Drug Administration, and the Israeli Ministry of Health Division of Inspection and Enforcement. I will also discuss a new test card for quantitative analysis of beta lactam antibiotics; this card could be used to detect substandard or degraded medications even if there is no giveaway signal from an unapproved excipient. The role of inexpensive screening tests as the top of a "funnel" for monitoring very low quality drugs globally will be discussed.

1315

A COHORT STUDY ON BREASTFEEDING AND EARLY INFANT FEEDING PRACTICES IN THE FIRST SIX MONTHS OF LIFE IN FORTALEZA, CEARÁ, BRAZIL

Bruna L. Maciel[1], Milena L. Moraes[2], Noélia L. Lima[2], Alberto M. Soares[2], Isabelle F. Cruz[2], Cláudia B. Abreu[3], Richard L. Guerrant[4], Aldo A. Lima[3]

[1]Universidade Federal do Rio Grande do Norte, Natal, RGN, Brazil, [2]Federal University of Ceara, Fortaleza, Brazil, [3]Federal University of Ceara and MAL-ED Network, Fortaleza, Brazil, [4]University of Virginia and MAL-ED Network, Charlottesville, VA, United States

Nutritional transition occurs in Brazil, and contrasting trends such as the co-existence of obesity/overweight and anemia are found in our population. The determinants of these trends in children may be associated with early and poor complementary feeding in the first months of life. This study aimed to describe breastfeeding, feeding practices and nutritional status in early childhood in a community from northeast Brazil. A cohort of the 6 first months of life of 242 children was conducted from November, 2010 to February, 2013. Exclusive breastfeeding was received by 64.5% of the children in the first month of life and only 4.8% in the 6th month of life. Complementary feeding was early offered to children: 9.5% received grain derived foods, 15.3% were feed with infant formulas and 13.1% with other milks in the first month of life. We observed increases in z scores for weight-for-age, length-for-age and weight-for-length during the follow up. The prevalence of high weight-for-length and high weight-for-age was 18.9% and 14.9% in the 6th month of life. Nevertheless, at 7 months of age, 42.1% of children had hemoglobin levels under 11mg/dL. The reduction in exclusive breastfeeding during the 6 months of study was associated with the prevalence of high weight-for-length (Chi-squared, $p < 0,0001$). Data is consistent with the nutritional transition phenomena occurring in Brazil and shows the need for public policy focusing on overweight and healthy feeding practices.

1316

ROSIE THE SPRAY TEAM LEADER, EXPANDING OPPORTUNITIES FOR WOMEN IN MALARIA PREVENTION

Jennifer Burnett, Jane Coleman, Nicole de Gier

Abt Associates, Bethesda, MD, United States

The President's Malaria Initiative (PMI) currently conducts indoor residual spraying (IRS) in 12 countries in Africa. Local country teams of 15-20 full time staff members in each country organize the spray campaign and hire hundreds or even thousands of seasonal employees to serve in roles ranging from spray operators to storekeepers and supervisors, all of which are essential to a successful spray campaign. Traditionally, women have been under represented in the IRS workforce and have generally been employed in lower level positions, such as washers. Four gender assessments were conducted in Ethiopia, Rwanda, Ghana, and Senegal in 2013 in order to better understand the number and type of positions that women held in IRS campaigns, identify gender-related challenges and constraints, and suggest areas for improvement. The assessments included focus group discussions, interviews, and an analysis of spray operations. The results indicate that women experience cultural, structural, and social challenges when joining the IRS workforce. Such challenges include women's lack of self-determination in regards to their participation in the spray campaign and perceptions that women are not physically fit enough to be spray operators. PMI has increased women's participation in IRS by specifically targeting them for recruitment through meetings held at the community level and adapting information, education, and communication materials to incentivize women to join the IRS workforce. As a result of these assessments and efforts to increase the number of women employed, and to ensure that they are employed in higher level positions such as spray operators and storekeepers, women currently hold 25% of IRS positions on average. Out of the thousands of workers trained for the 2012 and 2013 Rwanda spray campaigns, 26% and 31% (respectively) were women. This percentage increased to 50% of participation for women in the most recent 2014 spray campaign. This presentation will detail how IRS programs are working toward the proven and achievable goal of equal gender participation in other African countries who conduct IRS.

1317

EXPANDING HEALTH MINISTRY CAPACITY TO DELIVER MALARIA AND OTHER HEALTH COMMODITIES AT THE COMMUNITY LEVEL IN NIGERIAN STATES

William R. Brieger[1], **Bright C. Orji**[2], Emmanuel Otolorin[2], Hajara John Moses[3], John Orok[4]

[1]The Johns Hopkins University, Baltimore, MD, United States, [2]Jhpiego, an Affiliate of The Johns Hopkins University, Abuja, Nigeria, [3]Bauchi State Ministry of Health, Bauchi, Nigeria, [4]Akwa Ibom State Ministry of Health, Uyo, Nigeria

The highly participative process of community directed interventions (CDI) was first pioneered in 1996 by the African Program for Onchocerciasis Control for the delivery of ivermectin. CDI was further tested and found effective in delivering other health commodities. In 2007 Jhpiego began a proof of concept project in Akwa Ibom State, Nigeria and learned that CDI could be a useful vehicle for increasing access to and coverage of malaria in pregnancy interventions. Building on this success, Jhpiego expanded this work to include integrated community case management of malaria, diarrhoea and pneumonia. through community led efforts. The World Bank Malaria Booster Program, observing Jhpiego's efforts in Akwa Ibom State, asked the Nigeria National Malaria Control Program to enlist Jhpiego's help in building the capacity of seven State Ministries of Health (MOH) to organize CDI for what was termed the malaria plus package consisting of community case management and health promotion activities. The scale-up process started with workshops for state CDI implementation teams consisting of staff from malaria control and primary health care in the MOHs. Then these state teams developed their own intervention packages and organized workshops for local government teams, who in turn trained staff from their front line health facilities. These facility staff mobilized communities in their facility catchment areas (wards) to select volunteers for training on the CDI process and intervention package. Although technical assistance was provided to each state, challenges arose including commodity supplies and coordination among different program units within the state MOHs. In conclusion, state teams can train local government teams, ultimately cascading CDI to the community in order to scale up maternal and child health interventions.

1318

INFRASTRUCTURE INDEPENDENT POINT-OF-CARE MOLECULAR DIAGNOSTICS FOR LOW-RESOURCE SETTINGS

Robert A. Burton, Bernhard Weigl, David Boyle, Jered Singleton, Dylan Guelig, Steven Diesburg, Paul LaBarre

PATH, Seattle, WA, United States

In low-resource settings (LRS), limited access to centralized medical facilities presents a critical barrier to timely diagnosis, treatment, and related control and elimination of infectious diseases. Inadequate diagnostic laboratory infrastructure results in increased costs, lost test results, delays, and loss to follow-up associated with specimen transportation to health centers and subsequent response. At the same time, the most accurate diagnostic tests with low limits of detection (LODs) and high clinical sensitivity and specificity are only available through laboratory-based testing and more recently through portable nucleic acid amplification tests (NAATs). Indeed, NAATs are becoming increasingly important to identify and prevent transmission from asymptomatic infections (e.g. malaria) using active infection detection interventions in eliminating countries. NAATs are similarly important for early infection detection scenarios such as early infant or acute case detection (e.g. HIV). Unfortunately, many NAAT approaches are still tied to laboratory-style equipment and instrumentation such as heat blocks, centrifuges, optical detectors, and refrigerated cold-chain logistics, and therefore have limited reach. Additional hurdles must be overcome when considering specimen acquisition and lysis, and nucleic acid extraction and purification sufficient for subsequent amplification and detection in a NAAT. Isothermal amplification NAATs seem to address some low-resource requirements by obviating the need for thermal cycling and improved enzyme tolerance to inhibitors, reducing sample purification requirements. To date, appropriate, portable, rapid, infrastructure-independent, highly accurate NAATs that meet the WHO ASSURED guidelines remain elusive. In this presentation, we will address important considerations for the utilization of molecular diagnostics in LRS and present recent advancements from PATH's product development partnerships toward increasing access to sample-to-results NAATs in remote communities with limited resources, electricity, and infrastructure.

1319

SHARE YOUR FINDINGS: A GUIDE FOR SCIENTISTS AND MEDIA PROFESSIONALS TO GENERATE PUBLIC INTEREST IN RESEARCH

Debora Miranda[1], Douglas Hopper[2]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Bill & Melinda Gates Foundation, Seattle, WA, United States

Global health research aims to address the inequalities in health and improve the lives of populations at risk. But are research outcomes being effectively communicated to those who can put them into practice? An increasing number of funders see the value in allocating resources (budget and staff time) to the dissemination of research and results. Media professionals - from press and communications officers at research institutions to broadcast, print or online journalists - can make an important contribution in bridging the gap between academia and communities affected by global health issues. But many scientists still find themselves feeling frustrated about their work being simplified when it is communicated to wider audiences. On the other hand, the open access movement and the social media revolution are paving the way for scientific knowledge to be broadly publicised. This presentation will share lessons learned by representatives involved in the collaborative process of pitching and disseminating research, with the aim to increase collaboration and best practice. These include communications professionals from research institutions and a research funding organisation, an editorial member of a major peer-reviewed scientific journal, a journalist who has reported on and from an endemic country and a global health scientist with experience in translating research findings into policy.

1320

DE NOVO MICROSATELLITE MARKER MINING FROM SCARCE AMOUNTS OF *CULICOIDES* GENOMIC DNA: PATHWAY TO UNDERSTANDING DISPERSAL AND POPULATION OF THE VECTOR OF OROPOUCHE VIRUS

Maria Gorreti Onyango

Australian Animal Health Laboratory, Geelong, Australia

Oropouche virus is a member of the family Bunyaviridae and the cause of an important arboviral disease in South America. Since its first isolation in 1955, the virus has caused more than 30 epidemics and half a million infections. *Culicoides paraensis* is the major vector of Oropouche virus in urban epidemics of the disease. Dispersal and isolation levels of the different vector populations are key factors for spread of the pathogen and the potential vector control tools. Populations genetic studies of *C. paraensis* will be facilitated by improved genomic resources of the vector. Microsatellites are among the most informative and frequently used genetic markers. Their novel isolation from non-colonizable organisms and with limited quantity of genomic DNA (such as *Culicoides* vectors) can be a major challenge. Identifying effective means of increasing the amount of DNA for de novo microsatellite isolation from *Culicoides* spp. will facilitate study of their genetic variability and adaptation. *C. brevitarsis* is a known vector for bluetongue virus in Australia. Its genomic size overlaps that of *C. paraensis*. DNA from two pools of 15 female *C. brevitarsis* was amplified using the multiple displacement amplification technique. This was subsequently sequenced on ¼ picotitre plate of 454 GS FLX Roche sequencer. A total of 120 005 reads was obtained,

2594 putative microsatellite repeats were isolated from the raw reads using Msatcommander 8.0.2 program. 528 primers were designed to the flanking regions of the microsatellite repeats using primer 3 software. A fraction of these primer pairs were selected for validation. Eight of the primer pairs that amplified 100% of the populations have successfully been used to genotype 96 individuals from two populations of *C. brevitarsis*. This study has been able to overcome huge technical constraint due to the very tiny size of this vector and has developed technical workflow easily translatable to *C. paraensis*, an important vector of Oropouche virus.

1321

COMMUNITY BASED INDOOR RESIDUAL SPRAYING THE TOOL FOR REDUCING COST AND COMPLEXITY OF IRS: A PILOT STUDY IN TANZANIA

Rubona M. Joshua, Rubona M. Joshua

RTI - International, Mwanza, United Republic of Tanzania

Tanzania mainland has implemented indoor residual spraying (IRS) using different operational designs, starting with highly centralized (2007-2009) and medium decentralized (2010-2013). These two approaches were perceived to be complex to manage and expensive. We report a pilot of community based IRS (CBIRS) which is less complex, relatively cheaper and more community owned. CRIRS was organized and implemented at the village level, including: recruitment of spray operators by village governments; use of bicycles by spray operators for transportation; consent by village government to implement IRS; and construction of effluent waste disposal structures using local materials. To evaluate CBIRS pilot, focus group discussion were undertaken with Regional and District IRS technical teams, site managers, sub-site supervisors and Team Leaders, Village mobilizers and Site based mobilizers, Spray operators and community leaders. The evaluation also reviewed IRS implementation guide, IRS performance report, IEC meeting minutes, supervisors report and undertook inspection of constructed sub-sites for compliance to environmental requirements. The evaluation suggests that objectives of CBIRS were attained. CBIRS reduced implementation cost; increased community participation and ownership; reduced organizational complexity of IRS; achieved acceptable quality and quantity of IRS; and maintained compliance to environmental protection requirements. The evaluation revealed aspects that need improvement: training of team leaders was inadequate to cover their important roles in CBIRS; village mobilizer and sub-site supervisor were redundant; effluent disposal sites were unnecessarily large compared to small number of spray teams in CBIRS; and installation of two soak-pits was unnecessary as one pit can accommodate the small amounts of effluent waste generated by a small team.Community based IRS is an ideal approach to reduce cost and complexity of implementing IRS in Tanzania. Some modifications need be considered which include; omitting unnecessary roles like village mobilizer and sub-site supervisor; simplify fabrication of effluent waste disposal structures; and increasing the level of team leaders' training.

1322

NOVEL DETECTION OF *CARDINIUM* ENDOSYMBIONT IN *CULICOIDES* SPECIES

Peter T. Mee[1], Ary A. Hoffmann[2], Andrew R. Weeks[2], Peter J. Walker[1], Jean B. Duchemin[1]

[1]CSIRO Australian Animal Health Laboratory, Geelong, Australia, [2]Pest and Disease Vector Group, Bio21 Institute and the Department of Genetics, University of Melbourne, Melbourne, Australia

Culicoides are blood-sucking midges identified as one of the most significant genera in the family Ceratopogonidae, due to the ability to transmit a diverse range of pathogens. *Culicoides* serve as vectors of medically significant viruses, such Oropouche virus, and transmit a range of nematodes species including *Mansonella perstans* which causes perstans filariasis in Africa and South America. *Culicoides* also transmit a range of viral and filarial pathogens of veterinary importance. Global occurrence, capability of rapid and widespread dispersal and lack of effective control options makes *Culicoides* a major risk factor for the introduction and spread of these pathogens. In recent years, the characterization and use of endosymbiotic bacteria for the prevention of mosquito-transmission of pathogens has proved to have a high success rate in the laboratory. The most predominant example of this being the transinfection of *Wolbachia* into the dengue mosquito vector *Aedes aegypti* and the subsequent blocking of the mosquito's ability to transmit dengue virus. *Wolbachia* and *Cardinium* are both naturally occurring bacterial endosymbionts which infect *Culicoides*. There is currently a lack of information on the distribution and occurrence of these bacterial endosymbionts within these insects and their effects, if any, on pathogen transmission. This study has profiled the distribution of *Culicoides* species in south-eastern Australia and developed a range of screening assays to detect low level infections and explore the distribution of these endosymbionts. We have identified *Cardinium* infection in a range of *Culicoides* species including some of the most significant vector species. Sequence analysis has revealed that this is the same *Cardinium* species which is infecting multiple *Culicoides* species from a range of geographical locations including Japan, Israel, Madagascar, Australia and Africa. Experiments are currently underway to determine the potential influence that *Cardinium* infection may have on the host *Culicoides*. The identification and profiling of the endosymbiont *Cardinium,* could provide the first step towards endosymbiont-based control of these significant vectors of both medically and veterinary important pathogens.

1323

GLUTAMATE-GATED ION RECEPTORS IN THE TSETSE FLY *GLOSSINA MORSITANS MORSITANS*

Paul O. Mireji

KARI-Trypanosomiasis Research Institute, Nairobi, Kenya

Insect glutamate-gated receptors include ionotropic receptors (IRs) that mediate detection of volatiles, ionotropic glutamate receptors (iGluRs) and metabotropic glutamate receptors (mGluRs) which mediate impulse transmission across synapses. The IRs, are expressed in antennal coeloconic sensillae neurons, while iGluRs and mGluRs are expressed on post-synaptic membranes in central nervous system. In order to identify the *Glossina morsitans morsitans* IRs, iGluRs and mGluRs, known homologs from *Drosophila melanogaster* and ab initio approach based on glutamate-gated channel specific domains were used to search *Glossina* genome assembly and transcriptome Yale strain GMOY1.1. Phylogenetic relationships among *Glossina* IRs, iGluRs and mGluRs and their drosophila and anopheles homologs were determined using Maximum Likelihood estimates, and numerical relationships with selected diverse species gleaned from Phylome database. Relative expression levels among *Glossina* IRs, iGluRs and mGluRs were established using RNA-seq data of adult female fly. Overall, 40 putative glutamate-gated receptor loci comprising 19, 15 and six IRs, iGluRs and mGluRs respectively were recovered in *Glossina*. The *Glossina* iGluRs and mGluRs had higher sequence conservation than IRs relative to drosophila homologs. The *Glossina* IRs lacked at least one glutamate interacting residues except IR8a and IR25a, which showed high sequence similarity to iGluRs. Relative to D. melanogaster, annotation of *Glossina* revealed lower numbers of IRs, but certain loci had multiple related copies. The iGluRs numbers were similar, while mGluR-like loci were more. Suggestively, *Glossina* over-invests specific IR gene lineages for odor detection, but broadens odor-space discrimination in CNS. There was no glutamate-gated homolog of IR93a recoverable in *Glossina*. Apparently, there were three species-specific divergent IRs, perhaps relating to *Glossina*'s stable host range, thus reducing the range of odors to sample unlike other diptera. Because glutamate-gated receptors mediate rapid neuronal communications, they could be perfect targets for manipulation towards improving tsetse control tools.

1324

TARGETING EDUCATIONAL CAMPAIGNS FOR PREVENTION OF VECTOR-BORNE DISEASE: AN ASSESSMENT OF RURAL VS. URBAN SETTINGS IN THAILAND

Macy Brusich[1], John Grieco[2], Theeraphap Chareonviriyaphap[3], Nicole Achee[1]

[1]University of Notre Dame, Notre Dame, IN, United States, [2]Uniformed Services University of Health Sciences, Bethesda, MD, United States, [3]Kasetsart University, Bangkok, Thailand

Vector-borne diseases, such as malaria and dengue fever, are transmitted to humans by mosquitoes. Thailand, an endemic country for both of these diseases, serves as a platform to characterize the relationship between household vector control practices and individual health-seeking behavior. Such studies can guide educational campaigns for the awareness and prevention of disease as this relationship may vary between rural and urban settings. The overall goal of this study was to assess differences between knowledge, attitude, and practice (KAP) in persons presenting to health clinics with malaria and/or dengue fever manifestations in two distinct study sites in Thailand for the purposes of identifying key variables at the individual and household level that influence health behavior related to the prevention of vector-borne disease. Specific methodologies included a survey questionnaire performed at healthcare facilities followed by household mosquito collections and house structure characterization. Analyses will include whether or not the presence of mosquitoes, perception of exposure to mosquitoes and/or current acceptance and uptake of mosquito control practices at the household level differs between rural and urban study sites. Field activities will be completed July 2014 to be presented to the Ministry of Health of Thailand to serve as a guide for enhanced targeting of educational campaigns for the prevention of vector-borne diseases.

1325

EMERGING RESEARCH ON DIPTERA AS MECHANICAL VECTORS: THE CASE OF *BACILLUS ANTHRACIS*

Tatiana Eversley[1], David Adams[2]

[1]AASU, Savannah, GA, United States, [2]AASU/Medical College of Georgia, Savannah, GA, United States

Flies (Order Diptera) are well known for their role as mechanical vectors for enteric pathogens. Recently, however, researchers have found that certain flies, e.g., the Bluebottle Blow Fly (*Calliphora vicina*), the common house fly (*Musca domestica*), and the stable fly (*Stomoxys caltitrans*) are efficient mechanical vectors for *Bacillus anthracis*. Moreover, investigators have concluded that flies helped to trigger *B. anthracis* outbreaks that spanned not only neighboring districts but also international borders. This presentation will examine not only recent developments regarding the role of flies in anthrax outbreaks but also recommend possible prevention strategies.

1326

SCIENTISTS, PUBLICS AND TRANSGENICS: INFORMATION, TRUST, COMMUNICATION AND ENGAGEMENT ON RESEARCH DEALING WITH VECTOR-BORNE DISEASES

Christophe Boete[1], Uli Beisel[2], Luisa Reis Castro[3], Nicolas Cesard[4], Guy Reeves[5]

[1]Institut de recherche pour le développement, Marseille, France, [2]University of Bayreuth, Bayreuth, Germany, [3]Massachusetts Institute of Technology, Cambridge, MA, United States, [4]Kyoto University, Kyoto, Japan, [5]MPG-Plön, Plön, Germany

Infectious diseases transmitted by mosquitoes represent a burden for a variety of countries and especially for the Global South. However research aiming at better understanding them is mainly conducted by institutions from the Global North. Apart from bringing knowledge in biology, this research is obviously associated with the development of methods aiming at reducing the burden of vector-borne diseases and this includes the creation, the use and the release of transgenic mosquitoes. For many in the scientific world, this technological approach offers a promising method against diseases such as malaria or dengue. However the recent field releases of transgenic mosquitoes in The Cayman Islands, in Malaysia and in Brazil have been the source of intense debate in the specialized press as well as in the non-specialized mass media. This lack of transparency, not to say the secrecy, in the way the first trial was conducted is without much doubt the major reason for the controversy that emerged. Brushing aside years of discussion in the scientific world and a shared recognition of the importance to consider ethical, legal and social issues this first trial could be read as a fait-accompli: the cage of transgenic mosquitoes has now been opened. In the complex interactions between science and society around GM technology we cannot avoid questions around the perception of the public by scientists and the related question: How to consult, involve and engage a variety of publics in an effective manner on science and technology? With the will to better estimate the impact of geographic differences (endemic vs non endemic countries), of research topics (work on transgenic approach or not) and of perception of research (applied/ fundamental) we have conducted in 2012/ 2013 a worldwide web-based survey on more than 1800 scientists working on vector-borne diseases. This work reveals several interesting points including the reluctance in involving the public upstream, some lack of confidence in private business as well some level of distrust towards biotechnological progress and the speed at which changes occur because of science and technology. Surprisingly it also highlights a real lack of communication even inside the scientific community.

1327

GENETIC DIVERSITY AND POPULATION STRUCTURE IN THE *LEISHMANIA* VECTOR *LUTZOMYIA (NYSSOMYIA) ANDUZEI* (DIPTERA: PSYCHODIDAE) FROM THE BRAZILIAN AMAZON

Vera M. Scarpassa, Adrya da Silva Figueiredo, Ronildo Baiatone Alencar

Coordenacao de Biodiversidade, Instituto Nacional de Pesquisas da Amazonia, Manaus, Brazil

Lutzomyia anduzei has large geographic distribution in northern South America. This species has been implicated as a secondary vector of *Leishmania guyanensis*, the etiological agent of cutaneous leishmaniasis, in the Brazilian Amazon. In despite of possible involvement of *L. anduzei* in the leishmaniasis transmission, its biology and ecology are poorly known and none population genetics study was performed with this species. We sequenced 74 specimens of six *L. anduzei* localities from the Brazilian Amazon by analyzing 1201 base-pairs of the *COI* gene (mtDNA) to assess genetic diversity and population structure. The genetic diversity was fairly high with 58 haplotypes. Although none haplotype was shared among the localities, all haplotypes were connected in the network. The genetic diversity intra-population was fairly high for all samples (h = 0.859 to 1.000; π = 0.00601 to 0.01008). Values of pair-wise F_{ST} had a large range from 0.042 to 0.413, which were statistically significant (P<0.0001) for the most of comparisons. Similarly, the hierarchical analysis was highly significant among samples (F_{ST} = 0.166; P<0.0001), and the sequence divergence ranged from 0.75 to 1.30%. These results suggest that populations of *L. anduzei* consist of very high genetic variability; however, the gene flow was reduced among populations analyzed that resulted in moderate to large genetic structure. These findings may be implication in the transmission of *Leishmania* and in the control efforts across its range.

1328

DOG SEROLOGY FOR CUTANEOUS LEISHMANIASIS IS ASSOCIATED WITH SAND FLY VECTOR ABUNDANCE AND SUGGESTS ENDEMIC TRANSMISSION IN RURAL PANAMÁ

Luis F. Chaves[1], Azael Saldana[2], Indra Rodriguez[3], Kadir Gonzalez[2], Chystrie Rigg[2], Vanessa Pineda[2], Marcia D. Laurenti[4], Jose E. Calzada[2]

[1]Nagasaki University, Nagasaki, Japan, [2]Instituto Conmemorativo Gorgas de Estudios de la Salud, Panama, Panama, [3]Universidad de Panama, Panama, Panama, [4]Universidade de Sao Paulo, Sao Paulo, Brazil

American Cutaneous Leishmaniasis (ACL), beyond a neglected tropical disease, is a zoonosis, where several vertebrates can be infected by *Leishmania* spp parasites. The body of evidence supporting a reservoir role for dogs (*Canis familiaris*) remains contradictory, and it is unclear whether dogs have become major reservoirs in eco-epidemiological settings that have undergone major ecological transformations. Between April and June of 2010, we studied canine ACL in 52 dogs belonging to 24 households in Trinidad de Las Minas, Western Panamá. We collected information on potential ecological (domestic animal abundance, wildlife animal species diversity among others, vegetation, peridomiciliary structure and housing quality), entomological (sand fly abundance) and epidemiological (human infections) risk factors at the household level, as well as, blood samples and information on the health status of each individual dog. Blood samples were employed for *L.* spp/ *L. panamensis* PCR, ELISA and IFAT diagnostics. Bayesian evaluation of the serodiagnostics in absence of a gold standard, showed ELISA to be the most sensitive (0.79) and specific (0.84) diagnostic. ELISA based canine ACL seroprevalence was 47%. At the household level we found *Lutzomyia trapidoi* was the main risk factor for ELISA seropositive reactions (ESR) in dogs, increasing the odds ratio (OR) 2.28 by each sand fly caught inside the households/trap-night (SFA). At the individual level the OR of dog ESR increased 3.39 and 1.35 times by each SFA and year of age, respectively. Finally, the age specific ELISA based canine ACL seroprevalence curve allowed the estimation of a basic reproductive number (R_0 ± S.E.) of 1.22 ± 0.09 which indicates that canine ACL is endemically established in dogs at our study site. Our data suggest: i) that dogs are likely an incidental *Leishmania panamensis* host where LCA is endemically established and ii) that R_0 estimates from serological surveys should be interpreted cautiously, since they are only a robust indicator for endemic establishment in a focal population.

1329

SLEEPING HABITS AFFECT ACCESS TO HOST BY CHAGAS DISEASE VECTOR *TRIATOMA DIMIDIATA*

Etienne Waleckx, Rafael Pasos-Alquicira, Maria Jesus Ramirez-Sierra, Eric Dumonteil

Universidad Autonoma de Yucatan, Merida, Yucatan, Mexico

In the Yucatan peninsula (Mexico), the causative agent of Chagas disease *Trypanosma cruzi* is transmitted by the bug *Triatoma dimidiata*. While *T. dimidiata* invades and colonizes houses in other regions, this species has an intrusive behavior in Yucatan, probably attracted by artificial light and potential hosts, but has limited ability to establish colonies. Bugs collected inside the homes also have a low nutritional status, suggesting that they cannot efficiently feed inside these houses. We hypothesized here that this low feeding status of *T. dimidiata* may be associated with the local practice in Mayan communities to sleep in hammocks instead of beds, as this sleeping habit could be an obstacle for triatomines to easily reach their host, particularly for nymphal instars which are unable to fly. To test this hypothesis, we used an experimental chamber of 100 cm x 50 cm x 50 cm in which we placed a miniature bed in one side and a miniature hammock on the other side. After placing a mouse enclosed in a small cage in the bed and another one in the hammock, *T. dimidiata* specimens were released in the chamber and their activity was video recorded (7 pm-7 am). Our results show that bugs were similarly attracted to both mice in the bed and in the hammock. However, they were able to reach the mouse located in the bed significantly more frequently than that located in the hammock. Adults reached the bed most frequently by walking, while they reached the hammock most frequently by flying. Interestingly, nymphs were also able, in few occasions, to reach the mouse in hammock by walking. Our conclusion is that sleeping in hammocks as in rural Yucatan makes the host less accessible to triatomines and may explain, at least in part, the low nutritional status and limited colonization of houses by *T. dimidiata* in the region.

1330

ONCHOCERCIASIS TRANSMISSION IN GHANA: EFFECT OF VECTOR SPECIES ON HOST-SEEKING BEHAVIOR AND ONGOING TRANSMISSION

Poppy H. Lamberton[1], Robert A. Cheke[2], Martin Walker[1], Rory J. Post[3], Peter Winskill[1], Daniel A. Boakye[4], Mike Y. Osei-Atweneboana[5], Lee J. Crainey[6], Michael D. Wilson[4], María-Gloria Basáñez[1]

[1]Imperial College London, London, United Kingdom, [2]University of Greenwich of Medway, London, United Kingdom, [3]Liverpool John Moores University, London, United Kingdom, [4]Noguchi Memorial Institute for Medical Research, Legon, Ghana, [5]Water Research Institute, Accra, Ghana, [6]Fiocruz Amazônia, Manaus, Brazil

The World Health Organization has set goals for the control and elimination of human onchocerciasis by 2020 in selected African countries. The feasibility of achieving this depends on the initial level of onchocerciasis endemicity in the communities, the levels of geographical and therapeutic coverage and treatment compliance, and the patterns (intensity and seasonality) of transmission, including the species composition of the simuliid vectors and host-seeking behaviour. Ghana is renowned for its sibling species diversity of the *Simulium damnosum* complex, vectors of *Onchocerca volvulus*. Ghana was originally a country within the umbrella of the Onchocerciasis Control Programme in West Africa (OCP), initially a vector control programme, which operated between 1974 and 2002. We present the spatial and temporal patterns in transmission of *Onchocerca* spp larvae of host-seeking and ovipositing adult parous female flies in communities in Southern Ghana located inside and outside the prior OCP that have been treated with ivermectin for different durations. To date, results include monthly biting rates (MBR) ranging from 714 bites/person/month at Agborle Kame (100% *S. damnosum* s.str./*S. sirbanum* in the savannah region) to 8,586 bites/person/month at Pillar 83/Djodji (98.5% *S. squamosum* in the forest mosaic). MBRs were higher in the wet season. In contrast, parous rates were higher in the dry season (41.8% vs. 18.4%), resulting in higher monthly parous biting rates in the dry season. Monthly infectious biting rates ranged from zero to 79.4 infectious bites/person/month. Monthly transmission potentials ranged from zero to 794.3 infective larvae/person/month. Results will be presented in relation to density of vector and host species and the on-going transmission of *O. volvulus* having been used to parameterise EpiOncho models on the effect of vector species on transmission. Our results show that host choice varies between cytospecies, and may be affected by vector and/or host density with epidemiological relevance for vector-borne disease models.

1331

CIRCULATING ANTIBODY ISOTYPES IN SCABIETIC PATIENT SERA DIRECTED AT MITE ANTIGENS

Marjorie Morgan[1], Larry Arlian[1], Hermann Feldmeier[2]

[1]Wright State University, Dayton, OH, United States, [2]Charité University Medicine Berlin, Berlin, Germany

Scabies, caused by the mite *Sarcoptes scabiei*, is a worldwide neglected disease, particularly in limited-resource settings. The mite has developed resistance to the topical acaricides commonly used to treat this disease. In its early stages, scabies is difficult to diagnose. A focus of our research is to identify molecules that potentially could be used in developing a

diagnostic test for scabies and in a vaccine for its prevention in highly susceptible populations. A confounding problem is that scabies mites are the source of many antigens that cross-react with antigens of the ubiquitous allergy-causing house dust mites *Dermatophagoides farinae, D. pteronyssinus* and *Euroglyphus maynei.* We used an isotype-specific ELISA to screen serum collected from > 100 ordinary scabies patients against extracts of *S. scabiei*, *D. farinae, D. pteronyssinus* and *E. maynei.* At the time of diagnosis, most of these ordinary scabies patients exhibited circulating antibody to scabies antigens with IgG being the predominant isotype. Most patients also had circulating antibodies that bound to antigens from the *Dermatophagoides* mites. The most striking observation was that high scabies antibody titers were paralleled by high levels of antibody that recognized antigens from *E. maynei.* This was especially clear in the case of IgM, the first isotype produced in response to a foreign antigen. The results of this study further demonstrate that the cross-reactivity among mite antigens must be considered as diagnostic tests and vaccines for scabies are developed.

1332

ACCURATE SPECIES IDENTIFICATION AND PHYLOGENETIC RELATIONSHIPS REVEALED BY DNA BARCODING OF PERUVIAN SAND FLIES

Chukwunonso Onyemaechi Nzelu[1], Abraham G. Cáceres[2], Ken Katakura[1], Yoshihisa Hashiguchi[3], Hirotomo Kato[1]

[1]Laboratory of Parasitology, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan, [2]Departamento Académico de Microbiología Médica, Facultad de Medicina Humana, Universidad Nacional Mayor de San Marcos, Peru, and Laboratorio de Entomología, Instituto Nacional de Salud, Lima, Peru, [3]Department of Parasitology, Kochi Medical School, Kochi University, Japan, Prometeo, Secretaria Nacional de Educacion Superior, Ciencia, Tecnologia e Innovacion, Ecuador, and Centro de Biomedicina, Universidad Central del Ecuador, Quito, Ecuador

Phlebotomine sand flies (Diptera: Psychodidae) are the putative vectors of leishmaniasis worldwide. A reliable species identification of these minute insects constitutes the first step in the surveillance and control of leishmaniasis in endemic areas. Identifying sand fly species based on morphological characteristics is difficult and often complicated by phenotypic plasticity and cryptic species complex as well as demanding considerable taxonomic expertise. The use of DNA barcodes has been proposed recently as a tool for identification of the species in many diverse groups of animal. We assessed the utility of DNA barcoding, based on cytochrome c oxidase subunit I (COI) sequences, for identifying sand fly species from areas where leishmaniasis is endemic in Peru. A total of 89 sand fly specimens belonging to 16 morphological species and 2 genera - Lutzomyia and Warileya, including the major disease vectors were analyzed. We were able to recover and align the target COI fragment from all sand fly species we examined. Phylogenetic analysis of the sequences indicates that the observed species groupings were in confirmation with the morphological identification. The results obtained shows that the barcoding gene was useful in species discrimination in sand flies from Peru.

1333

DEVELOPMENT OF A NOVEL ASSAY TO MEASURE FLIGHT CAPACITY OF *ANOPHELES GAMBIAE* S.L.

Amy R. Krystosik[1], **Diana L. Huestis**[2], Tovi Lehmann[2]

[1]Kent State University, Kent, OH, United States, [2]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

Anophelines are important vector species in sub-Saharan Africa and contribute to the continued transmission and burden of malaria worldwide. The dry-season ecology of anophelines, specifically in the arid Sahel conditions, remains unknown, but two hypotheses have been proposed to explain the repopulation phenomenon after the dry season: aestivation and migration. To investigate the migration hypothesis, we developed an activity meter to measure flight by sound accounting for environmental conditions. We found that intensity of sound can predict flight density at frequency of 400-800 Hz; however, this was only achievable at temperatures greater than 63°C in the G3 colony. A second stimulant used to induce flight was patchouli; but, due to background noise in the lab, we could not detect change in intensity by cage density although relative observed flight did increase with cage density. Further work can expand this activity meter to a flight to exhaustion assay which is currently under development. These methods may be field-adaptable, allowing us to study if is it possible that mosquitoes repopulate by migration.

1334

ADULT *AEDES AEGYPTI* SURVEILLANCE USING THE BG SENTINEL TRAP IN PHNOM PENH, CAMBODIA

Michael Prouty[1], Didot B. Prasetyo[1], Rekol Huy[2], Noch Nin[1], Sam Bunleng[2], Ngan Chan Sophal[2], Ngan Chantha[2]

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

Adult mosquito surveillance was conducted in Phnom Penh, Cambodia using BG sentinel traps (Biogents AG) from October 2012 through October 2013. Traps were set indoors in 18 volunteers' houses around Phnom Penh (two collection sites for every district). Traps were set for a 72 hour collection period per month and mosquitoes were collected every 24 hours from the traps. Habitat variables at each collection site such as premise condition index, presence of paddy fields in the surrounding area, mosquito control effort, and house density were measured. Mosquito specimens were transferred to laboratory and identified to species level. The relationship between weather variables (rainfall, near surface temperature, and specific humidity) and *Aedes aegypti* abundance was measured to determine weather's influence on mosquito population. In total 15,536 mosquitoes, representing 20 species in 9 genera were collected. The predominant species were *Culex quinquefasciatus* (76.57%), *Ae. aegypti* (12.93%), and *Anopheles vagus* (7.03%). *Cx. quinquefasciatuis* the primary vector for *Wuchereria bancrofti* and *Ae. aegypti* for dengue and Chikungunya viruses. Weekly accumulated rainfall (mm) was positively correlated with *Ae. aegypti* abundance at three weeks time lag (P=0.004) while monthly near surface air temperature (°C) was positively correlated at one month time lag (P<0.001). However, no positive correlation was found between specific humidity and *Ae. aegypti* abundance. Monte Carlo permutation test showed that mosquito population was significantly correlated to the presence of paddy fields in the surrounding area (P=0.001), mosquito control effort (P=0.004), and house density (P=0.048). Canonical Correspondence Analysis (CCA) showed that the presence of *Ae. aegypti* was postively associated with house density, and negatively associated with paddy field presence and mosquito control effort at collection sites.

1335

MISUSE AND ABUSE OF ANTIMICROBIALS: COULD WE BE SUPPORTING MALARIA PARASITE DEVELOPMENT IN THE MOSQUITO HOST?

Jewelna Osei-Poku[1], Mathilde Gendrin[2], Dorothy Yeboah-Manu[1], George K. Christophides[2], Michael D. Wilson[1]

[1]Noguchi Memorial Institute for Medical Research, Legon, Accra, Ghana, [2]Division of Cell and Molecular Biology, London, United Kingdom

Naturally-occurring bacteria inhabiting the guts of mosquito vectors are important determinants of vector competence; some species can effectively kill ingested parasites, thus reducing disease transmission. Treating mosquitoes with antibiotics/antimicrobials clears the bacteria in the gut allowing for enhanced development and transmission of parasites.

We hypothesize therefore, that the overuse of antibiotics/antimicrobials among human populations may inadvertently impact on the efforts to control malaria transmission. Experiments that have shown the effect of bacteria clearance of *Plasmodium* have used high concentrations of antibiotics/antimicrobials which may not reflect levels that circulate in the human serum. This study seeks to investigate the effect of human serum concentrations of commonly administered antibiotics/antimicrobials on the gut microbiota of *Anopheles gambiae* s.l, and the consequential effect on *Plasmodium falciparum* development. Preliminary results have been obtained from the initial phase of this project which involves determining the core gut microbiome of *Anopheles gambiae* s.l. sampled from Accra, Ghana. DNA from guts of 66 female adults reared from a field collection of larvae and pupae were analyzed using 454-pyrosequencing. Using the Mothur and QIIME software, preliminary results showed the gut microbial community were predominantly *Gammaproteobacteria* (98.5%); *Enterobacter* (24.8%), *Klebsiella* (21.7%), *Serratia* (39.2%) and *Stenotrophomonas* (2.1%) species. This differs from what has been reported in *Anopheles gambiae* from Kenya, which comprised of mainly *Thorsellia* (67.6%) and *Propionibacterium* (9.08%). Further studies will investigate the effects of varying levels of commonly administered antimicrobials on the *An. gambiae* gut microbiota and the consequential effects on *P. falciparum* development. The results from this study are expected to inform on possible negative effect of the unbridled use of antimicrobials on the control of malaria.

1336

YEAST SYMBIONTS IN ARTHROPOD VECTORS: POSSIBLE IMPLICATIONS FOR THE CONTROL OF VECTOR-BORNE DISEASES

Sara Epis[1], Guido Favia[2], Irene Ricci[2]

[1]Universita' Degli Studi di Milano, Milano, Italy, [2]Universita' Degli Studi Di Camerino, Camerino, Italy

The increased awareness for the environmental and the public health problems caused by the use of chemical compounds to combat vector-borne diseases (VBDs) is leading to the development of alternative control strategies. Biological control of arthropod vectors and VBDs is generally based on the use of bacteria, such as *Bacillus thuringiensis*. Although bacterial symbioses in arthropod vectors are the focus of several research programs aimed at developing strategies to control VBDs, such as malaria, dengue, and trypanosomiasis, arthropod-associated yeasts have not yet been deeply investigated. Here we present the first results of a long-term project, aimed at developing strategies for the control of VBDs, exploiting yeasts associated with arthropod vectors. The first disease vectors discovered to harbor yeast symbionts are mosquitoes, from the genera *Anopheles* and *Aedes*. Yeasts isolated from these mosquitoes have been identified as *Wickerhamomyces anomalus*, a typical killer yeast characterized by a wide-spectrum antimicrobial activity, including the production of killer toxins (KTs). Several *W. anomalus* are already used as biopreservation agents in the control of post-harvest diseases of vegetables. The antimicrobial activity of the *W. anomalus* isolated from mosquitoes has been tested *in vitro* against sensitive microbes, showing that these mosquito-associated yeasts actually release an effective antimicrobial KT. Further to mosquitoes, we are currently screening different arthropod vectors such as ticks and send flies for the presence of killer yeasts. Other experiments are aimed at determining whether killer yeasts and their toxins modulate arthropod immunity. Killer yeasts could thus be exploited for a double action, a direct anti-pathogenic effect within the vector and an immune stimulation, with an indirect effect on the reduction of the vectorial capacity. We expect that our project will increase the knowledge on this different type of symbionts, and to the development of novel tools for the biological/integrated control of vector-borne tropical diseases.

1337

HYPOTHESIS TESTING CLARIFIES *TRIATOMA DIMIDIATA* (LATREILLE, 1811) SYSTEMATICS USING NUCLEAR ITS-2 AND MITOCHONDRIAL CYTOCHROME B GENES

Patricia L. Dorn[1], Nicholas de la Rua[1], Heather Axen[2], Nicholas Smith[2], Bethany R. Richards[1], Jirias Charabati[2], Julianne Suarez[1], Dulce M. Bustamante[2], Carlota Monroy[3], Johana Maradiaga[1], C. William Kilpatrick[2], Eric Dumonteil[4], Lori Stevens[2]

[1]Loyola University New Orleans, New Orleans, LA, United States, [2]University of Vermont, Burlington, VT, United States, [3]Universidad de San Carlos, Guatemala City, Guatemala, [4]Universidad Autonoma de Yucatan, Merida, Mexico

The widespread and diverse *Triatoma dimidiata* is the species most important for Chagas disease transmission in Central America and an important vector in Mexico and northern South America. Its diversity may contribute to different Chagas disease prevalence in different localities and has led to conflicting systematic hypotheses describing various populations as subspecies or cryptic species. To resolve these conflicting hypotheses, we sequenced a nuclear (ITS-2) and mitochondrial gene (cytochrome *b*) from an extensive sampling of *T. dimidiata* across its geographic range. We assessed the congruence of ITS-2 and cyt *b* phylogenies and tested the statistical support for constrained topologies representing competing systematic hypotheses. Unconstrained phylogenies inferred from ITS-2 and cyt *b* are congruent. However, hypothesis testing does not support the division of *T. dimidiata* into the previously proposed three sub-species inferred from morphology and ITS-2. Our results identify two cryptic species and indicate *T. dimidiata* sensu stricto is not subdivided into monophyletic clades that might indicate subspecies. Extensive specimen sampling, analysis of both a hypervariable mitochondrial gene and a slower evolving nuclear gene in conjunction with statistical tests of hypotheses has facilitated the clarification of evolutionary relationships among epidemiologically important populations of *T. dimidiata*.

1338

ANTIBACTERIAL ACTIVITY FROM EXTRACTS OF FATTY BODIES AND HEMOLYMPH OF THE BLOWFLY *SARCONESIOPSIS MAGELLANICA* (DIPTERA: CALLIPHORIDAE)

Felio J. Bello[1], Jennifher Gongora[1], María A. Gaona[1], Jesús A. Cortes-Vecino[2]

[1]Universidad del Rosario, Bogotá, Colombia, [2]Universidad Nacional de Colombia, Bogotá, Colombia

Sarconesiopsis magellanica is a necrophagous and hemisynanthropic fly which belongs to the Calliphoridae family. Its importance for human and veterinary medicine lies in its potential for participating as mechanical vector for pathogens such as viruses, bacteria, fungi, protozoa and helminths. Its larvae could cause miasis in some vertebrates, including human beings. Moreover, this fly is used in determining the post-mortem interval. Taking into account its necrophagous habits, this fly could be considered as a potentially useful model in larval therapy. The main goal of this work was to evaluate the antibacterial activity of the extracts of fatty bodies and hemolymph from third-instar larvae of *S. magellanica*. The results were compared with the effects obtained from the same substances derived of the blowfly *Lucilia sericata*, under *in vitro* conditions. The fatty bodies of larvae were removed by dissection technique and the hemolymph via decapitation and centrifugation of larval specimens. The tested bacteria were *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The methods used to evaluate the antibacterial activity were agar diffusion and colony forming units. After accurate incubation, the results showed that the antibacterial activity of fatty bodies in both *S. magellanica* and *L. sericata* were effective against *S. aureus* and *P. aeruginosa*, but there was not significant difference between the fly species. However, in the agar diffusion assay the antibacterial activity of the extracts of fatty bodies of both species was found to be more efficient against *P. aeruginosa.*

The obtained results suggest that these substances could have a similar effect against the evaluated microorganisms in the treatment of infected wounds.

1339

NICHE CONSERVATISM AND PHYLOGENETIC STRUCTURE IN BROAD-SCALE SPECIES RICHNESS PATTERNS OF CHAGAS DISEASE VECTORS

Carlos N. Ibarra-Cerdeña[1], Fabricio Villalobos[2], Janine M. Ramsey[3]

[1]Centro de Investigaciones y de Estudios Avanzados del IPN, Unidad Mérida, Mérida, Mexico, [2]Universidade Federal de Goiás, Goiânia, Brazil, [3]Centro Regional de Investigaciones en Salud Publica, Instituto Nacional de Salud Publica, Tapachula, Mexico

A major concern in evolutionary ecology and biogeography is the study of species distribution, especially when species from the same genus have a phylogenetic structure showing non-random spatial association. This pattern can emerge from historical processes as phylogenetic relationships and niche conservatism. In species with public health importance, such as insect vectors, looking into these patterns is of great relevance since processes keeping or avoiding the phylogenetic structure can be key factors in developing control and prevention measures and to anticipate measures to mitigate global change effects. Here, we used simulation models to analyze species richness patterns in the Triatominae (Reduviidae) based on collection data points, species distribution models, climate and phylogenetic information. Patterns of simulated co-distribution and co-diversity under different hypothesis were compared with empirical models. We found that historical processes as phylogenetic relationships and niche conservatism are important causes shaping current patterns of species richness. We consider that our approach has a broad application in quantitative biogeography of vectors of other diseases.

1340

DEPLETION OF TICK THIOREDOXIN REDUCTASE ATTENUATES THE NATIVE TICK MICROBIOTA

Khemraj Budachetri, Shahid Karim

The University of Southern Mississippi, Hattiesburg, MS, United States

The gulf-coast tick (*Amblyomma maculatum*) is a competent vector for a variety of pathogenic microbes, including *Rickettsia parkeri*, a causative agent of Spotted Fever Rickettsiosis. Ticks experience a variety of oxidative stress condition while on and off the vertebrate host. To counter-act the deleterious effects of reactive oxygen species, ticks have numerous antioxidant molecules in their repertoire, such as the Thioredoxin-Thioredoxin Reductase (Trx-TrxR) system, as reported previously. Tick Thioredoxin Reductase has barely been investigated. Our long-term goal is to reduce or block the spreading of vector-borne pathogens by interfering with vector proteins. In this study, we tested our hypothesis that tick TrxR facilitates the colonization of microbes in tick tissues by mitigating the reactive oxygen species. Transcriptional gene expression studies examining the level of TrxR during the prolonged blood-meal in both midguts and salivary glands indicates a potential need of this system during unfed stage. In order to evaluate the functional significance of this highly conserved system, we utilized RNA interference to selectively deplete TrxR transcripts *in vivo*. Both transcriptional gene expression and enzymatic activity studies confirmed the successful depletion of TrxR transcript and activity. However, no significant effect was observed on total tick engorgement likely due to high redundancies or compensatory mechanism in ticks but, the tick salivary glands super oxide dismutase (SOD) was found similarly down regulated with the TrxR depletion. Disruption of TrxR reduces the microbial load in the salivary glands examined by using bacterial universal 16s rRNA gene primers. Our results support the potential role of TrxR in preserving bacterial communities in tick tissues by alleviating the deleterious effect of reactive oxygen species. This work opens new avenues of research in oxidative stress within tick vectors and vector-borne pathogens.

1341

VECTORBASE: A BIOINFORMATICS RESOURCE CENTER FOR INVERTEBRATE VECTORS OF HUMAN PATHOGENS

Gloria I. Giraldo-Calderon[1], Daniel Lawson[2], Scott J. Emrich[1], Frank H. Collins[1]

[1]University of Notre Dame, Notre Dame, IN, United States, [2]EMBL-EBI, Hinxton, United Kingdom

The VectorBase database is updated and expanded every two moths. In the last year we have significantly updated the gene builds, the assemblies or both for *Glossina morsitans, Aedes aegypti*, *Rhodnius prolixus, Anopheles stephensi* and *An. darlingi*. We have also almost tripled the number of hosted genomes from 11 to 30. These new genomes include the two sandflies *Lutzomyia longipalpis* and *Phlebotomus papatasi*, 16 new *Anopheles* species, and the snail *Biomphalaria glabrata*, an intermediate host of *Schistosoma mansoni*. Based on user feedback and internal discussions, all of our tools and resources have had multiple interface and performance improvements, including the possibility to save and reuse job parameters and their results. A new tool called the Population Biology Browser (PopBio), which we had presented initially under a beta version, was also released. This new tool is part of our ongoing efforts to integrate genomic, phenotypic (including insecticide resistance) and population data, as a strategy to integrate basic and applied research. VectorBase also includes new ontologies, mitochondrial sequences, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway information, microarray experiments, single-nucleotide polymorphisms (SNPs) data, RNAseq experiments and other datasets that are available for query and analyses. Also under development is the new VectorBase Galaxy Platform, which will provide our community with a user-friendly interface to perform large scale data analysis on a public site. The data deposited in VectorBase and in the public repositories such as NCBI, are a resource that has been subject to only very limited preliminary analysis. These data are freely available for new analyses, descriptions and hypotheses testing.

1342

SURVEYING THE BACTERIAL COMMUNITY PROFILES IN TICKS FROM THE VILLAGES OF INDIGENOUS PEOPLE IN MALAYSIA

Jing-Jing Khoo, Kai-Ling Kho, Chee-Sieng Khor, Syuhaida Sulaiman, Kim-Kee Tan, Azzy Izyati Ahmad Shanizza, Sun-Tee Tay, Sazaly Abu Bakar

University of Malaya, Kuala Lumpur, Malaysia

Ticks are excellent vectors for disease transmission of a wide variety of zoonotic pathogens, including viruses, bacteria and protozoa. In our laboratory, we are interested to explore the microbiome in ticks collected from the villages within the semi-forested areas in Malaysia. These areas are known as the interfacial zones of inhabitants (IZI), which provide for plenty of opportunities for contact between the indigenous people occupying the villages, tick vectors and wildlife reservoir hosts harbouring an array of zoonotic pathogens. Hence, the indigenous people in the IZI are constantly at threat from tick-borne diseases due to close contact with the tick vectors. We aim to utilize the 16S ribosomal RNA metagenomic sequencing strategy as a means to investigate the bacterial community in ticks collected from IZI, in hope of identifying emerging pathogens in ticks from IZI. Our results indicate that there is prevalence of a number of tick-borne bacterial pathogens harboured by the ticks sampled from the IZI. As the knowledge and data on pathogens harboured by ticks in Malaysia is minimal, studying the bacterial community in ticks, together with clinical surveillance, will provide knowledge that may help in the early detection of emerging pathogens among the indigenous people in Malaysia.

1343

FINE SCALE MAPPING OF QTL ASSOCIATED WITH REPRODUCTIVE DIAPAUSE WITHIN THE *CULEX PIPIENS* COMPLEX USING A RADTAG GENOMIC APPROACH

Roberta Engel, David W. Severson

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

Culex pipiens is a broadly distributed species complex that transmits human diseases (e.g., West Nile Virus, Lymphatic Filariasis). *Cx. p. pipiens*, one member of the complex, is found across temperate zones of the world while *Cx. p. quinquefasciatus* is restricted to subtropical and tropical regions. One physiological trait that distinguishes *Cx. p. pipiens* from its sister taxon is its ability to enter reproductive diapause. Photoperiod is the primary trigger of this complex life history trait. Previous work using markers developed with traditional methods inferred four quantitative trait loci (QTL) in an F2 mapping population. The ability to generate informative Single Nucleotide Polymorphic markers (SNPs) and infer QTL has increased dramatically with the advent of massively parallel sequence technology (e.g., Illumina HiSeq2000). In addition, a published reference genome for *Cx. p. quinquefasciatus* is available. An advanced intercross line (*Cx. p. quinquefasciatus* Johannesburg x *Cx. p. pipiens* South Bend) was established. First instar larvae collected from the F6 generation were exposed to diapause inducing conditions (i.e., 8:16 light:dark cycle and 18C). Follicle size in ten-day old adult females was used to score phenotype. Only the extreme phenotypes were sampled to construct a reduced representation paired-end library. Using a RADtag approach, each of the 100 samples had a unique identifier. SNPs were generated *in silico*; a filtered subset of 2000 SNPs was used to infer linkage groups. Linkage groups with at least 15 markers were assigned to chromosomes. Marker density on the linkage map is an order of magnitude greater than on the map used in an earlier study. Presently we are mapping QTL regions on a fine scale. This has positioned us to advance our understanding of what genes and genetic pathways regulate reproductive diapause.

1344

PHYSICAL MAPPING REVEALS CHROMOSOME-SPECIFIC GENOMIC LANDSCAPES IN *ANOPHELES STEPHENSI*

Ashley Peery, Xioafang Jiang, Brantley Hall, Maria Sharakhova, Zhijian Tu, Igor Sharakhov

Virginia Polytechnic Institute and State University, Blacksburg, VA, United States

Anopheles stephensi type form is the key vector of malaria on the Indian subcontinent and the Middle East. Additionally, *An. stephensi* is an emerging model species for genetic and genomic studies of mosquito biology and mosquito-parasite interactions. However, success of genomic analyses will be limited if researchers deal with numerous sequencing scaffolds, rather than with a chromosome-based genome assembly. Here we report the first chromosome-based genome assembly for the Indian wild type strain of *An. stephensi*. Our physical chromosome mapping ordered 62% of the *An. stephensi* sequencing scaffolds and facilitated analysis of chromosome arm-specific genomic landscapes that is seldom feasible in next-gen genome projects. Comparative analysis between *An. stephensi* and *An. gambiae* revealed differences in genome organization and highlighted varying rates of evolution between autosomes and the sex chromosome. The genome landscape of *An. stephensi* is characterized by relatively low repeat content compared with that of *An. gambiae*. Our analysis demonstrated extremely high rate of rearrangements in the X chromosome as compared with autosomes despite the lack of polymorphic inversions in the X chromosomes in both species. Additionally, the difference between the rates of the X chromosome and autosome evolution is much more striking in *Anopheles* than in *Drosophila*. We found that the high rates of evolution in the X chromosome highly positively correlated with the density of simple repeats, suggesting their role in genomic plasticity. While, the rate of autosomal evolution and distribution of common polymorphic inversions positively correlates with the densities of microsatellites and genes, but negatively correlates with the coverage of matrix associated regions and transposable elements. Our data indicate that overall high rates of chromosomal evolution are not restricted to Drosophila, but may be a feature common to Diptera. The chromosome-based genome assembly for *An. stephensi* will provide a valuable tool for the vector biology community as we seek a better understanding of mosquito biology.

1345

SILENCING CASPASE DECREASES DENV-2 INFECTION OF THE MOSQUITO VECTOR *AEDES AEGYPTI*

Matthew W. Eng, David W. Severson

Eck Institute for Global Health, Department of Biological Sciences, Notre Dame, IN, United States

The mosquito *Aedes aegypti* is the primary vector for dengue virus (DENV). An understanding of host-pathogen interaction is important in understanding what factors contribute to vector competence. However, many of the molecular mechanisms for vector competence remain unknown. Our previous global transcriptional analysis has suggested the induction of apoptotic proteins in the involvement of resistance and susceptibility to DENV-2 infection. Here we analyze the possibility that programmed cell death is actively involved in the defense of *A. aegypti* host cells to DENV-2 infection. The initiator caspase, Dronc, has been previously shown to be an essential component of the core apoptotic pathway. This caspase showed higher expression *in vitro* in infected *A. aegypti* cells as well as in resistant mosquitoes following infection. However, TUNEL staining of midguts from DENV-2-resistant and -susceptible mosquitoes revealed that apoptosis is activated at near-basal levels early during infection. Interestingly, dsRNA interference of Dronc decreased virus titer and infection in resistant mosquitoes. This reveals that Dronc may be important for affecting DENV-2 infection in *A. aegypti*. Furthermore, we investigate whether silencing of Dronc effects non-apoptotic processes influencing DENV-2 infection.

1346

POPULATION OF *LUTZOMYIA LONGIPALPIS* (DIPTERA: PSYCHODIDAE: PHLEBOTOMINAE) FROM PANAMA

Anayansi Valderrama Cumbrera[1], Jose Dilermando Andrade Filho[2], Mara Garcia Tavares[3]

[1]Gorgas Conmemorative Institute, Panama, Panama, [2]Instituto René Rachou-Fiocruz, Belo Horizonte, Minas Gerais-Brasil, Brazil, Brazil, [3]Universidade Federal de Viçosa, Brazil, Brazil

Although many studies on vectors of cutaneous leishmiansis have been done in Panama, the perdomestic distribution of *Lutzomyia longipalpis* have been documented recently. With the purpose of estimated the divergence time and difference in genetic population between peridomestic and selvatic species of *Lu. longipalpis*, we performed this study to estimate and compare the intra- and inter-population genetic variability of wild and near-residential populations of *Lu. longipalpis*, obtained in a locality with high incidence of cutaneous leishmaniasis in Panama. Using a mitochondrial DNA sequences of cytochrome B were analyzed of *Lu. longipalpis* populations from Panama. Of the 11 haplotypes obtained, seven were present exclusively in the town of El Limón and three, exclusively in Bona Island. A single haplotype was shared between the two communities. The haplotype and nucleotide diversities were h=0.70 and π=0.0015 for the population of Bona and h=0.95 e π=0.003 for the population of El Limón. The genetic differentiation analyses between the two populations showed significant differences (Fst=0.17; $p<0.05$) between them. Significant differences ($p<0.05$) were also obtained when the Panama sequences were compared to others obtained in Genebank cytochrome B the populations of *Lu. longipalpis* from Colombia (Fst=0,98), Costa Rica (Fst=0,98), and Brazil (Fst=0,72). The existence of unique haplotypes in each community and the significant genetic differentiation reported suggest that the *Lu. longipalpis*

populations in Panama are in the middle of a speciation process due to the isolation of the two populations because of the Pacific Ocean and the events that characterized the emergence of the Isthmus of Panama. The fact that *Lu. longipalpis* was found in near residential areas in Panama is important as a risk factor and to increase epidemiological surveillance. We result indicate the need to constantly and systematically monitor of this vector species in regions with high incidence of leishmaniasis and review the symptoms produced by different cryptic species of *Lu. longipalpis*. Meanwhile, little is currently known about the distribution, occurrence, and implications of this species in the transmission of leishmaniasis in the country.

1347

BIONOMICS AND PHYLOGENETICS OF THE DENGUE VECTOR *AEDES AEGYPTI* FROM THE ARABIAN PENINSULA

Emad I. Khater[1], Farrukh Baig[1], Amgad A. Saleh[1], Kashif Munawar[1], Hany A. Kamal[2]

[1]College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia, [2]Ain Shams University, Research and Training Centre on Vectors of Diseases, Cairo, Egypt and Dallah Corp., Jeddah, Saudi Arabia

Aedes aegypti is the principal vector of dengue in the world, including Saudi Arabia and Yemen in the Arabian Peninsula south-western regions; where disease outbreaks have occurred since 1995. Understanding the ecology and population genetics of *Ae. aegypti* is crucial for understanding dengue virus transmission patterns and for effective disease control. We report here on the ecology and phylogenetics of *Ae. aegypti* collected from western Saudi Arabia, from Jeddah governorate, a major harbour on the Red Sea. Phylogenetics analysis was carried out using the ribosomal DNA-internal transcribed sequence 2 (ITS2) and the mitochondrial cytochrome *c* oxidase I (COI) and NADH dehydrogenase subunit 4 (ND4) genes. *Aedes aegypti* larvae and pupae collected represented 23.9% (n= 772: 712 larvae, 60 pupae) of the total culicines mosquitoes collected. Most of water sites were anthropogenic, of which plastic drinking water tanks were the most productive for larvae (av. 55.5±55.5 larva/site). The most productive sites for pupae (47.5% of total pupae) were large concrete underground tanks or plastic elevated tanks (1000-5000 L capacity). The pupal yield is much lower than those reported from other countries. Single nucleotide polymorphisms (SNPs) and Neighbour-Joining (NJ) phylogenetic trees were built using COI and ITS2 sequences obtained from *Ae. aegypti* from Saudi Arabia or retrieved from the Genbank for other populations from Africa, Asia and the Americas. NJ trees identified ten COI and 21 ITS2 haplotypes, with many haplotypes unique to Arabian *Ae. aegypti* populations. Data on ND4 analysis will be reported when appropriate. We provide novel phylogenetic information of *Ae. aegypti* populations from the Arabian Peninsula and other parts of the Oriental, Afrotropical and Palaearctic zoogeographic zones, which shows the presence of considerable genetic differentiation between them. These studies will give broader insights on the dispersal patterns of *Ae. aegypti* and transmission dynamics of dengue virus, with important implications for disease control under national and regional biogeographic conditions.

1348

MOLECULAR PHYLOGENETICS OF MOSQUITOES FROM THE ORIENTAL AND AFROTROPICAL ZOOGEOGRAPHIC REGIONS

Kashif Munawar, Amgad A. Saleh, Emad I. Khater

College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia

The Arabian Peninsula (PA) has peculiar position bordering the Oriental, Afrotropical and Palaearctic zoogeographic zones with diverse ecology and fauna. In Saudi Arabia (SA) (the largest country in PA) about 35 culicine mosquito species were reported including eight dominant vector species. The most important of these are *Aedes aegypti* and *Culex pipiens* complex, vectors of arboviruses, and two *Anopheles* malaria vectors, *An. stephensi* in Asia and *An. arabiensis*, the only member of *An. gambiae* complex outside Africa. We present here new information on phylogenetics of *An. stephensi* and *An. arabiensis* and other anopheline species collected from different SA regions. Neighbour-Joining (NJ) phylogenetics trees were constructed using DNA sequences of the ribosomal DNA-internal transcribed sequence 2 (ITS2) and the mitochondrial cytochrome *c* oxidase I (COI) gene. These sequences were obtained from mosquitoes field-collected from SA or from lab colonies from other countries in the Oriental or Afrotropical zones and sequences retrieved from the Genbank. Multi-locus phylogenetic analysis of COI & ITS2 sequences of all *An. stephensi* populations identified new haplotypes, including unique haplotypes to SA, and haplotypes broadly-distributed across the Oriental zone including AP. These results confirm that *An. stephensi* is a monophyletic species composed of ecotypes. However, unlike in Iran and India, we could not differentiate between *An. stephensi* type and mysorensis ecotypes, which might be due to inter-population extensive gene exchange. New *An. arabiensis* haplotypes were identified SA and related to field and lab populations from the Afrotropical region. In this report we provided new information on the phylogenetic relationships of anopheline mosquitoes from different zoogeographic regions including malaria vectors and suspected or non-vectors. Such information is important for understanding malaria transmission under broad biogeographic conditions across different zoogeographic zones. The COI or ITS2 sequences could also be used to develop species-specific molecular assays to complement pectorial keys to accurately identify species in AP and their cryptic or ecotypes if exist.

1349

POPULATION STRUCTURE OF THE VECTOR MOSQUITO *AEDES AEGYPTI* AND HUMAN-MEDIATED DISPERSAL IN THE PHILIPPINE ARCHIPELAGO

Eugenio Fonzi[1], Yukiko Higa[1], Arlene G. Bertuso[2], Emiko Kawashima[1], Kogomi Minagawa[1], Kyoko Futami[1], Jinping Hu[1], Noboru Minakawa[1]

[1]Institute of Tropical Medicine - Nagasaki University, Nagasaki, Japan, [2]University of the Philippines, Manila, Philippines

Aedes aegypti is the primary vector of most of the so-called arboviruses (ARthropod-Borne viruses), like dengue fever, yellow fever or chikungunya. Massive employment of insecticides favoured the development of insecticide resistance. *Ae. aegypti* is a highly anthropophilic mosquito and it is believed that human transportation played and still plays an important role in its dispersal. The dispersal ability of a vector is connected to its ability to spread the diseases as well as the insecticide-resistance mutations. Knowledge of the genetic structure of the populations of mosquitos can help to infer its patterns of dispersal. The Philippines are endemic for dengue fever and recently a high level of insecticide resistance was found. With its 7000 islands the philippine archipelago is therefore an appropriate environment to analyze the relationships between mosquito dispersal and both land and marine human transportation. With the objective of determining the distribution and population structure of Ae aegypti, during September-October 2013 a sampling took place in 7 major islands in the northern part of the Philippines (Luzon), in 11 seaports and 7 inland areas. In each area, at least 7 breeding sites were sampled; in order to reduce the presence of sibling individuals, (1) the flight range of *Ae. aegypti* was taken into account and (2) 1 out of 6 larvae collected from each site were randomly selected for the study, yielding between 19 and 67 individuals to be analyzed in each area. All the inland areas had to be discarded because of lack of specimen. Up to now, a preliminary analysis has been conducted with 6 microsatellite markers, but more are planned to be added henceforth. Between 4 and 9 alleles were found at each locus. Generally no significant deviation from HWE was found. The total Fst value was 0.06, quite low, suggesting gene flow between the islands. Interestingly, the pairwise Fst values were, at average, lower for the biggest and busiest seaports while higher for the smallest seaports.

1350

GENOME-WIDE HAPLOTYPE MAP REVEALS INSECTICIDE SELECTIVE SWEEPS IN WILD *ANOPHELES GAMBIAE* POPULATIONS FROM KENYA

Xiaohong Wang, Jun Li

University of Oklahoma, Norman, OK, United States

Nearly a million people die from malaria annually. *Anopheles gambiae* in Africa is the major malaria vector. Examining the molecular basis of mosquito traits of interests needs the information of genetic variations and haplotype map (HapMap) in wild *A. gambiae* populations from malaria endemic areas. We sequenced the genomes of nine wild *A. gambiae* individually, and detected 2,219,918 single nucleotide polymorphisms (SNPs) with 88% novel, and 43,765 nonsynonymous. SNPs are not distributed on *A. gambiae* evenly, and the lower SNP frequency regions overlays with heterochromatin and chromosome inversion. About 785,687 SNPs that were genotyped correctly in all individual mosquitoes with 99.6% confidence were extracted from high throughput sequencing data. Based on these SNP genotypes, we for the first time constructed the genome-wide HapMap of wild *A. gambiae* mosquitoes from malaria endemic areas in Kenya, and made it available through a public web with graphic user interface. Low LD is consistently observed with average linkage disequilibrium (LD) block size less than 40 bp. Meantime, we discovered that several large LD blocks were clustered in *A. gambiae* genome. Interestedly, detailed analysis of the genomic locus of chromosome 2 (2R:57.6-2L:4.0MB) that has fewer SNPs and largest linkage disequilibrium (LD) blocks revealed para gene at the center of this region with homozygous insecticide knock-down resistance (kdr) allele 1014F in all sample mosquitoes, supporting the hypothesis of insecticides DDT and pyrethroids selective sweeps in western Kenya.

1351

MOLECULAR ADAPTATION OF THE OLFACTORY SYSTEM TO HUMAN HOSTS IN *ANOPHELES GAMBIAE*

Giridhar Athrey[1], Theresa Hodges[1], Luciano Cosme[1], Willem Takken[2], Michel A. Slotman[1]

[1]Texas A&M University, College Station, TX, United States, [2]Wageningen University, Wageningen, Netherlands

The dominant African malaria vector *Anopheles gambiae s.s* preferentially takes it blood meals from human hosts, often at rates as high as 90%. This adaptation to human hosts is expected to have a genetic basis in the olfaction system, which includes several key gene families - the olfacation receptors (ORs), ionotropic receptors (IRs), odorant binding proteins (OBPs). We previously identified six narrow QTL for human host preference on chromosomes 2 and 3, that together explain 49% of the phenotypic variance. A total of 34 ORs, 7 IRs and 21 OBPs are located inside these QTL. In addition, a comparison of antennal transcriptomes identified 11 olfaction genes that are located inside QTL and that were significantly higher expressed in *An. gambiae* vs the zoophilic *An. quadriannulatus*. The genes involved in the adaptation of *An. gambiae* to human hosts should show evidence of positive selection. Therefore, we examined the evolution of olfaction genes (spanning all three gene families) from 95 individuals comprising five member species of the *An. gambiae* complex – *An. gambiae* (M + S), *An. arabiensis*, *An. melas*, *An. merus* and *An. quadriannulatus.* We used a phylogenetic framework (PAML) to test if the *An. gambiae* lineage evolved under positive selection– based on the ratio of non-synonymous to synonymous (dN/dS) substitutions, and signatures of selective sweeps. The presence of olfaction genes that evolved under positive selection inside human host preference QTL indicates their importance for human host choice in *An. gambiae.*

1352

UNEXPECTED STRONG REDUCTION OF GENE-FLOW WITHIN *ANOPHELES GAMBIAE* IN AN AREA OF HYBRIDIZATION WITH *AN. COLUZZII* IN THE "FAR-WEST" OF THEIR RANGE

Beniamino Caputo[1], Davis Nwakanma[2], Francesco Paolo Caputo[1], Musa Jawara[2], Cheryll Eniyou Oriero[2], Majidah Hamid-Adiamoh[2], Ibrahima Dia[3], Lassana Konate[4], Vincenzo Petrarca[1], Joao Pinto[5], David Conway[6], **Alessandra della Torre**[1]

[1]University of Rome Sapienza, Rome, Italy, [2]Medical Research Council The Gambia, Fajara, Gambia, [3]Institute Pasteur Dakar, Dakar, Senegal, [4]University of Dakar, Dakar, Senegal, [5]Universidade Nova de Lisboa, Lisbon, Portugal, [6]London School of Tropical Medicine & Hygiene, London, United Kingdom

The *Anopheles gambiae* complex includes mosquito species at different stages of speciation, ranging from clearly defined, although morphologically indistinguishable, bonae species to closely-related sympatric taxa such as *An. gambiae* and *An. coluzzii* (recently raised to formal species), which represent the major vectors of human malaria in sub-Saharan Africa. Extensive genetic studies have trusted these species as models of ecological speciation and highlighted the effect of this process in malaria epidemiology and control. We sampled *An. gambiae* and *An. coluzzii* populations from diverse habitats along the Gambia River (West Africa), an area characterized by higher level of inter-specific hybridization compared to most of the species range. We carried out a comparative analysis of these samples by presumably neutral nuclear microsatellite markers on chromosome-X and -3 and by presumably adaptive chromosomal paracentric inversions on chromosome-2. Both genetic markers reveal unexpected striking genetic differences, compatible with a strong reduction of gene-flow, between *An. gambiae* populations west and east of the central part of the transect, apparently exclusively colonized by *An. coluzzii*. While *An. gambiae* western populations are characterized by low chromosomal inversion diversity, a very high degree of chromosomal variation, based on a higher number of inversion polymorphisms, is observed in eastern populations. Consistent with this chromosomal divergence, high genetic differentiation at the microsatellite level, not explained by geographic distance alone, is observed between western and eastern populations. Notably, this microsatellite differentiation is higher than that observed between *An. gambiae* and *An. coluzzii*, and mostly due to loci in the centromeric region of chromosome-X. This suggests that the two *An. gambiae* populations may be at an advanced stage of reproductive isolation, likely triggered by human-made habitat fragmentation, and provides new evidence of a speciation continuum within the *Anopheles gambiae* complex.

1353

METAGENOMICS OF *AEDES ALBOPICTUS*: IMPACT OF LARVAL HABITAT TYPES AND MOSQUITO AGE ON THE MICROBIOME STRUCTURE OF MOSQUITO GUTS

Xiaoming Wang[1], Daibin Zhong[2], Thomas M. Gilbreath, III[2], Guofa Zhou[2], Tong Liu[1], Xiaoguang Chen[1], Guiyun Yan[2]

[1]Key Laboratory of Prevention and Control of Emerging Infectious Diseases of Guangdong Higher Education Institutes, Department of Pathogen Biology, School of Public Health and Tropical Medicine, Southern Medical University, Guangzhou, China, [2]Program in Public Health, College of Health Sciences, University of California Irvine, Irvine, CA, United States

Recent metagenomic studies suggest microbiomes of disease vectors may have profound impacts on vector development, reproduction, immunity against pathogens and vectorial capacity. However, the relationship between vector environments and vector microbiome structure and composition is unknown. Given mosquito larvae are confined to the aquatic habitats, it is hypothesized that microbial community in the larval habitats may largely determine the contents of mosquito larval guts, but larval gut microbials may have little effects on the gut microbial community of adult mosquitoes due to constant acquisition of new

microbials in the process of sugar and blood feeding. The present study tested this hypothesis with the Asian tiger mosquitoes (*Aedes albopictus*), a most invasive species and also an important dengue vector. We examined the dynamics of gut microbial communities of *Ae. albopictus* from three types of larval habitats, mosquito larvae, pupae and adults from these habitat types. Microbial community of the larval habitats and larval and adult mosquito guts was examined by pyrosequencing of bacterial 16S rRNA gene V4 hyper-variable region. A total of 15 million 250bp paired-end sequence reads were obtained. Preliminary analysis found that the composition of the microbiomes varied significantly among larval habitat types, and varied between larvae and adults whereas microbiomes of larvae and pupae were similar and resembled to the microbiomes of the larval habitats. *Proteobacteria*, *Bacterioidetes* and *Firmicutes* were the predominant bacteria across mosquito life stages. Blood feeding showed a significant impact on mosquito gut microbiomes. The present metagenomic study established a metagenomic foundation for better understanding the impact of environmental microbials on vector development and disease transmission.

1354

DISSECTING GENETIC AND MICROBIAL FACTORS OF *AEDES AEGYPTI* FIELD POPULATIONS WITH DISTINCT SUSCEPTIBILITY DO DENGUE VIRUS

Carine Spenassatto, Jaqueline Jarusevicius, Jayme A. Souza-Neto

São Paulo State University, Botucatu, Brazil

Dengue is the arboviral disease of highest public health concern due to its increasing expansion in recent years worldwide. Due to the lack of a licensed anti-dengue therapy, the prevention of dengue virus (DENV) dissemination is still limited to the control of its vector, the mosquito *Aedes aegypti*. DENV propagation and transmission is determined by the mosquito's vector competence, which has been associated to both genetic factors and the gut microbiota. Here we assess both the genetic variation of DVHFs (dengue virus host factors) and the microbial diversity of three field-derived Brazilian *Ae. aegypti* populations displaying distinct susceptibilities to DENV. Mosquitoes were collected in three different locations (Botucatu-SP, Neópolis-SE and Campo Grande-MS). We assessed dengue viral susceptibility of each population through oral infection by DENV-4 and quantified the relative number of viral particles by real-time PCR. Our data suggest that mosquitoes from Botucatu are nearly 3-fold less susceptible to the virus than those from Campo Grande ($p<0.001$). Sequencing analysis of the DVHFs lola and NADH of these two populations revealed a total of 9 SNPs, with 5 of them causing amino acid changes to the predicted polypeptide sequence of such genes. In order to verify a potential association between mosquito's microbial diversity and susceptibility to the virus, we are also performing Illumina 16S rRNA surveys to analyze the gut microbiota of such mosquitoes. Surprisingly, our results revealed that the midguts of the mosquitos from Botucatu are colonized mainly by Gram-positive bacteria from the Lactobacillus genus (34% of the total number of bacteria), even though there was a higher number of Gram-negative genera than Gram-positive ones in these mosquitoes. We are now assessing the Campo Grande population microbiome in order to determine whether the microbial diversity of these highly DENV-susceptible mosquitoes is different from that of the Botucatu population. This work will shed light on our understanding of the molecular interactions of DENV-mosquitoes-microbiota and may ultimately lead to the development of new dengue control strategies.

1355

GENOME-WIDE ISOLATION WITHIN THE WEST-AFRICAN MALARIA VECTOR *ANOPHELES MELAS*

Kevin C. Deitz[1], Giridhar Athrey[1], Musa Jawara[2], Hans J. Overgaard[3], The Anopheles Genomes Cluster Consortium[4], Michel A. Slotman[1]

[1]Texas A&M University, College Station, TX, United States, [2]Medical Research Council, Bakau, Gambia, [3]Norwegian University of Life Sciences, Ås, Norway, [4]University of Notre Dame, Notre Dame, IN, United States

Anopheles melas is a locally important malaria vector along the West-African coast where it breeds in brackish water. A recent population genetic study of this species revealed species-level genetic differentiation between two population clusters on the mainland: *An. melas* West and *An. melas* South. *An. melas* West extends from The Gambia to Tiko, Cameroon (near Mount Cameroon). The other mainland cluster, *An. melas* South, extends from the southern Cameroonian village of Ipono to Angola. Species level differentiation was also found between mainland and Bioko Island, Equatorial Guinea populations. To examine how genetic differentiation between these three forms is distributed across the genome, we pooled samples from a representative population of each of the three genetically isolated *An. melas* clusters. We performed whole genome sequencing on these pools and conducted genome-wide analyses of divergence and selection. Our analyses reveal that these three forms show high levels of genetic differentiation across the genome, including the presence of genome-wide fixed differences. Levels of genetic differentiation are particularly high on the *X* chromosome and low in heterochromatic regions. Additionally, we analyzed genome-wide differentiation between *An. gambiae* and *An. melas* West to put our results in the context of the *An. gambiae* species complex evolution. We also investigated how divergence in specific genes and genomic regions may have led to the genomic isolation of these putative species.

1356

MICRORNAS (MIRS): A VIABLE OPTION FOR TRANSGENIC MOSQUITO CONTROL?

Luciano V. Cosme, Craig J. Coates, Michel A. Slotman

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

MicroRNAs (miRs) are small non-coding RNAs that can each regulate the expression of up to hundreds of genes. Therefore miRs that are active during changes in host seeking behavior, as mosquitoes age or acquire a blood meal, may be a viable target for the transgenic manipulation of mosquitoes. However, the number of known miRs in the yellow fever mosquito is small compared to other insects and little is known about which genes they regulate. Because olfaction genes are crucial for host seeking, we examined if miRs play a role in olfaction gene regulation as mosquitoes develop and blood feed. We extracted total RNA from the antennae and head+thorax from females of various ages, as well as males (12h non-host seeking females, 4 days old host seeking females, 4 days old males, and 3h, 24h, 48h and 72h after blood feeding). Poly(A)+ RNA, 3' UTR and small RNA were sequenced on the Illumina platform. Global gene expression analyses revealed 52 genes that are highly and uniquely expressed in the antennae of 4 days old females (low or absent expression in 12h old females antenna, male antenna or head+thorax of 4 days old females). Similarly, 1,150 genes are uniquely expressed in the antenna of 12h old females. While 37 olfaction genes are differentially expressed between antenna of 12h old females and 4 day old females, only five of these are significantly different 24h after blood feeding in comparison to the 4 day old unfed females. The most expressed miRs in the antenna of females is aa-miR-236a, which has 82.65 fold higher expression compared to the head+thorax sample. We are particularly interested in miRs that do not kill mosquitoes, but decrease their attraction to humans. To identify miRs important for host seeking, we are injecting its antagomir (synthetic

anti-sense miR) into late larvae, pupa and 12h old adults. Preliminary results from pupa injection are very promising; injected mosquitoes are being subjected to a dual choice assay.

1357

ASSOCIATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN IMMUNE RESPONSE PATHWAYS GENES, AND SUSCEPTIBILITY/RESISTANCE PHENOTYPES OF *ANOPHELES DARLINGI* TO *PLASMODIUM VIVAX*

Nilton B. Rodrigues[1], Rejane C. Simoes[2], Nagila F. Secundino[1], Yoosook Lee[3], Wanderli P. Tadei[2], Paulo F. Pimenta[1], Gregory Lanzaro[3]

[1]Centro de Pesquisas René Rachou - FIOCRUZ, Belo Horizonte - MG, Brazil, [2]Laboratory of Malária and Dengue, Instituto Nacional de Pesquisas da Amazônia, INPA, Manaus, Brazil, [3]Vector Genetics Laboratory, University of California Davis, CA, United States

Populations of *Anopheles* malaria vector in nature are composed of both, *Plasmodium* susceptible and resistant individuals. Susceptibility and resistance to malaria parasites were, and still are, the subject of intense study. With the availability of *Anopheles darlingi* genome sequence, this new knowledge has opened doors for investigating genetic determinants for susceptibility to *P. vivax* infection of this major malaria vector in Americas. This work aims to describe the occurrence and distribution of SNPs (single nucleotide polymorphisms) in genes of the immune signaling pathways using samples taken directly from natural populations of *A. darlingi* (collected in Amazonas and Pará States in Brazil) and to investigate their association to susceptibility / refractoriness to *P. vivax* infections. We identified homologs of 172 immune genes in the *A. darlingi* genome from the data available on VectorBase. We conducted whole genome sequencing on 24 individuals both infected and uninfected groups and identified SNPs on immune genes. A SNP genotyping assay will be developed from a panel of non-synonymous SNPs in immune genes and genotyping assay will be conducted on 400 individuals of both infected and uninfected groups. Our goal is to identify SNPs associated with the susceptibility / resistance to *P. vivax* in *A. darlingi* populations of different genetic backgrounds. We will use the knowledge of the molecular genetics of *A. darlingi*, available on the Vector Base, to create and establish a database to be used in the recognition of genetic markers, which can be used as indicative of the existence of subpopulations of *A. darlingi* with distinct vector competence for transmission of human malaria in different localities of the Amazon. We intend to develop a predictive model of transmission that will point out where are the most competent mosquitoes population for the transmission of the parasite, which may help to establish strategies focused on the monitoring and control of the disease.

1358

IDENTIFICATION OF *ANOPHELES* (DIPTERA: CULICIDAE) FAUNA FROM COLOMBIA THROUGH DNA BARCODES

Giovan F. Gómez, Natalí Álvarez, Juan J. Quintero, Estefani A. Piedrahita, Margarita M. Correa

Universidad de Antioquia, Medellin, Colombia

Taxonomic determination of *Anopheles* species constitutes an essential baseline for targeted malaria vector control. Historically, morphological characters have been used for taxonomic identification; however, the existence of species complexes, closely related species and inter and intra phenotypic variation, makes this task difficult. Therefore, a DNA barcoding strategy based on a fragment of the *COI* gene has been proposed to identify specimens at the species level. In Colombia, approximately 47 *Anopheles* morphospecies have been recorded, however, molecular work has mainly focused on the main malaria vectors. The aim of this work was to provide a sequence reference library that includes DNA barcodes available for the corresponding Colombian species. In total 41 Molecular Operational Taxonomic Units (MOTUs) representing species/lineages were compiled, 30 of them were sequences obtained by our group or from GenBank. The remaining represented specimens from neighbor countries but that have also been recorded in Colombia. Neighbour-joining analysis based on Kimura's two parameter (K2P) showed non-overlapping clusters for all species and lineages with high bootstrap support, whereas similarity methods, Best Match, Best Close Match and All Species Barcode used with the typical 3% threshold proposed for barcode, correctly assigned 95.59% , 91.82% and 67.5% of the sequences respectively, to its original species. These results demonstrate that barcode constitutes an important tool for taxonomy in *Anopheles*; however, being a single-gene method its use constitutes a baseline approach, and other biological, morphological and ecological markers should be implemented for species delimitation. Importantly, the barcode sequence library presented here can be used as a benchmark for molecular confirmation.

1359

ENTOMOLOGICAL INVESTIGATIONS FOR UNDERSTANDING JAPANESE ENCEPHALITIS VIRUS TRANSMISSION DYNAMICS: LESSONS FROM BANGLADESH

Jennifer S. Lord[1], H. M. Al-Amin[2], Sumit Chakma[2], Shafiul Alam[2], Emily S. Gurley[2], Juliet R. Pulliam[1]

[1]University of Florida, Gainesville, FL, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

Understanding pathogen transmission dynamics is imperative for identifying and implementing cost effective interventions for optimal impact. One of the first steps toward understanding transmission dynamics of mosquito-borne zoonoses is to identify the host and vector species necessary for maintaining, amplifying and bridging transmission to humans. Such investigations were first undertaken for Japanese encephalitis virus (JEV) in Japan in the 1950's. Since this time, the dominant vector species – *Culex tritaeniorhynchus* – and reservoir hosts – pigs and ardeid birds – that were identified in these studies have generally been assumed to drive JEV transmission across the whole of Asia. This transmission cycle is likely to be responsible for human risk in areas where pigs are dominant within the community of vertebrate hosts and *Cx. tritaeniorhynchus*, confirmed in field and experimental settings to feed predominantly on large mammals, is relatively more abundant than other potential vector species. Such ecological contexts are found in Thailand and Malaysia; however, the presumption that this group of species drives transmission in all regions may impede our understanding of spatiotemporal variation in transmission dynamics of JEV. Countries where transmission drivers may differ from that of Japan include India, Indonesia and Bangladesh, where dead-end hosts (cattle) are found in substantially higher density than pigs. We utilize field data obtained during a preliminary entomological survey in Bangladesh to show that the observed dominance of any mosquito species within a community can be dependent on the sampling method employed. In addition we utilize an equation for the basic reproduction number of a zoonotic mosquito-borne virus, parameterized from field data and literature surveys, to demonstrate that the vector species observed to be most abundant may not necessarily drive transmission. To conclude, we emphasize that multiple, carefully selected mosquito sampling methods should always be considered for estimation of mosquito relative abundance as well as species blood-feeding patterns, when undertaking surveys to implicate vector and host species in new geographic regions.

1360

EPIDEMIOLOGICAL PATTERNS OF ROSS RIVER VIRUS DISEASE IN QUEENSLAND, AUSTRALIA, 2001-2011

Weiwei Yu

Queensland University of Technology, Brisbane, Australia

Ross River virus (RRV) infection is a debilitating disease which has a significant impact on population health, economic productivity and tourism in Australia. This study examined epidemiological patterns of

the RRV disease in Queensland, Australia between January 2001 and December 2011 at a statistical local area level (Figure 1). Spatial-temporal analyses were used to identify the patterns of the disease distribution over time stratified by age, sex and space. The results show that the mean annual incidence was 54 per 100,000 people, with a male: female ratio of 1:1.1. Two space-time clusters were identified: the areas adjacent to Townsville, on the eastern coast of Queensland; and the south east areas (Figure 2). Thus, although public health intervention should be considered across all areas in which RRV occurs, it should specifically focus on these high risk regions, particularly during the summer and autumn to reduce the social and economic impacts of RRV.

1361

RISK EVALUATION OF THE RIFT VALLEY FEVER EMERGENCE IN EUROPE: COMPETENCE OF THE EUROPEAN MOSQUITOES AND ADAPTABILITY OF THE VIRUS

Faustine Louis, Ilaria Castelli, Marie Vazeille, Anna-Bella Failloux

Institut Pasteur Paris, Paris, France

The Rift Valley Fever virus (RVFV) first detected in Kenya in 1930 causes a zoonose with an important impact on livestock. Very recently, it has expanded its natural range of distribution outside the Sub-Saharan Africa, in Saudi Arabia, Yemen, Madagascar, the Comoros and Mayotte islands. Its current expansion questions on the risk of a RVFV emergence in Europe. RVFV is an arbovirus with an enveloped particle composed of 3 negative single-stranded RNA segments which is transmitted by more than 30 different mosquito species. It circulates among wild mammals at a low prevalence but when environmental conditions are favorable for mosquito proliferation, an epidemic can occur causing mass abortions and death of young animals. Humans are mainly contaminated by direct contacts with tissues and blood when manipulating infected animals. Thus, the economic and social impacts of a RVFV epidemic can be dramatic. The aim of our study will be to evaluate the risk of RVFV emergence in Europe and the conditions that could favor its transmission. It will be done by developing two objectives: (i) determine the distribution and the competence to RVFV of potential mosquito vectors in France, and (ii) determine if molecular changes in the viral genome can be associated to an increased transmission by European mosquitoes?

1362

INSECTICIDE RESISTANCE STATUS IN *ANOPHELES GAMBIAE S.L.* FOLLOWING THE SCALE UP OF MALARIA CONTROL INTERVENTIONS IN RWANDA

Emmanuel Hakizimana[1], Gad Iranzi[1], Dunia Munyakanage[1], Rhodaly Muthoni[2], Corine Karema[1]

[1]Ministry of Health, Rwanda, Kigali, Rwanda, [2]Abt Associations Inc., Kigali, Rwanda

The scale up of malaria vector control interventions in particular universal coverage (one net for two persons) with Long Lasting Insecticidal Nets (LLINs) achieved in February 2011 and Indoor Residual Spraying (IRS) have played a major role in reducing by 86 % malaria incidence in Rwanda. The spread of insecticide resistance that has been reported in the African region may reverse the tremendous gains made in malaria control. Since 2010, the Malaria and Other Parasitic Diseases Division (MOPDD) in Rwanda has conducted resistance monitoring of malaria vectors to detect trends and to guide vector control interventions. Since 2010, resistance monitoring of malaria vectors was conducted in eight sentinel sites located in four provinces in Rwanda with varying malaria endemicity. The collection of *Anopheles* larvae in the field was conducted as described by WHO (2002) and reared to adults in controlled field conditions. In 2010, resistance testing was carried out using the CDC bottle assay. From 2011, WHO insecticide susceptibility testing was used (WHO 1998). The susceptibility outcomes were assessd according to WHO standard procedures (WHO, 2013). In 2010, resistance of *Anopheles gambiae s.l.* was only detected to DDT 4% in two (25%) out of eight sites surveyed. In 2011, resistance to DDT 4% was confirmed in four sites (28%) and emerging resistance to Permethrin 0,75% in three (21%) out of 14 sites. In 2012, the resistance was again confirmed to DDT 4% in one site (20%) and to pyrethroids in two sites (40%) out of 5 sites. Likewise, in 2013, the resistance to DDT 4%, Pyrethroids (Lambdacyalothrin 0,05%, Permethrin 0,75%, Deltamethrin 0,05% and Etofenprox 0,5%) and Bendiocarb 0,1% was respectively found out in eight (29%), fifteen (55%) and two (7%) sites out of 27 sites monitored. During this period, all specimen of *Anopheles gambiae s.l.* tested were susceptible to organophosphates (Fenitrothion 1% and Malathion 5%) at all sites. In conclusion, the scale up of malaria vector control interventions has associated with the spread of insecticide resistance of malaria vector mainly to pyrethroids. In response to this threat, an insecticide resistance management strategy was developed by the Ministry of Health of Rwanda and has to be regularly reviewed. Therefore, further investigations have to be undertaken to elucidate the resistance mechanisms.

1363

POPULATION DYNAMICS OF MAJOR MALARIA VECTORS AND THE IMPACT OF INDOOR RESIDUAL SPRAYING ON ENTOMOLOGICAL INOCULATION RATE IN NASARAWA STATE, NORTH CENTRAL NIGERIA

Petrus U. Inyama[1], Lazarus M. Samdi[1], Henry Nsa[1], Nduka O. Iwuchukwu[1], Lena Kolyada[2], Aklilu Seyoum[3], Dereje Dengela[2], Bradford Lucas[2], Funto Adewusi[1], Joseph Ifeanyi Okeke[1], Christen Fornadel[4]

[1]AIRS Project, Abuja, Nigeria, [2]Abt Associates, Bethesda, MD, United States, [3]AIRS Project, Ghana, Accra, Ghana, [4]United States Agency for International Development, Bureau for Global Health/HIDN, Washington, DC, United States

The President's Malaria Initiative | Africa Indoor Residual Spraying project, executed asecond year of spray operations in Nasarawa Eggon and Doma Local Government Areas of Nasarawa State, Nigeria. Molecular tools were used to identify the predominant vectors responsible for malaria transmission in the study area, and entomological inoculation rates (EIRs) were calculated pre and post intervention. Mosquitoes were identified morphologically and by molecular methods using polymerase chain reaction (PCR). The *Plasmodium falciparum* circumsporozoïte indexes were measured by ELISA and the EIRs were calculated for the 3 areas. A total of 2,539 *Anopheles* mosquitoes were caught in the intervention areas and control site. Of these, 1,653 (65.1%) were caught in Doma, 525 (20.7%) in Lafia and 361(14.2%) in N/Eggon respectively. A subsample of 1,265 *Anopheles* mosquitoes were randomly selected for PCR analysis. Morphological analysis indicated that 1,174 (92.8%) were *An. gambiae* s.l., while the remaining were *An. funestus* (3.6%), *An. pharoensis* (2.8%) and *An. squamosus* (0.8%). PCR analysis of the *Anopheles gambiae* s.l. revealed a predominance of *An. gambiae* s.s (68.5%), while 29.9% were *An. arabiensis*. ELISAs showed that *P. falciparum* sporozoite infection rates were 1.7% in *An. gambiae* s.s. and 0.6% in *An. arabiensis*. There was a significant difference between the sporozoite rate of *An. gambiae* s.s. and *An. arabiensis* (χ^2=8.696, p<0.0032, df=1). At baseline (pre-intervention), EIR was found to be 1.31infective bites/person/night (bpn) in Doma, 0.16 in N/Eggon and 0.13 in Lafia, including both indoor and outdoor collections. After the IRS intervention, EIR was reduced to 0.9 in Doma and 0.11 in N/Eggon, while it remained the same at the control area in Lafia at 0.13 bpn. There was a significant difference in EIR reduction (p<0.0001) between the intervention areas and the control site. Although ELISA tests incriminated *An. gambiae* s.s. as the predominant vector responsible for transmission of malaria in the study area, *An. arabiensis* was also found to be sporozoite positive. *An. funestus* group were not incriminated in malaria transmission. Post intervention EIRs were observed to have significantly decreased in the intervention areas. These findings provide information on the relative roles of the main malaria vectors found in the study areas and the impact of indoor residual spraying on malaria transmission.

1364

DYNAMIC RELATIONSHIPS BETWEEN MOSQUITO MICROBIOME AND VECTOR COMPETENCE

Brittany Dodson[1], Laura Kramer[2], Jason Rasgon[1]

[1]Pennsylvania State University, University Park, PA, United States, [2]New York State Department of Health, Wadsworth Center, Slingerlands, NY, United States

The hologenome theory of evolution proposes that natural selection of an organism is also driven by its symbiotic microorganisms. Research on the insect holobiome (the host plus all associated microorganisms) has largely been descriptive and often ignored when studying influences on phenotype. For insect vectors of medical importance, pathogen transmission capability is often variable, possibly due to differences in the internal host environment. Given that, functional knowledge about the holobiome of insect vectors is key to understanding vector-borne disease distribution and anticipating possible consequences of global climate change. Several studies have described mosquito symbionts and have suggested that microbe abundance and diversity can impact malaria parasites. However, the function and utility of those microbes are virtually unknown, especially in mosquitoes that transmit viruses. We compared microbiome data of two phenotypically distinct colonies of *Culex tarsalis* mosquitoes for West Nile virus (WNV) vector competence. Our data suggests that vector competence may be influenced by the mosquito microbiome and specific candidate microbes may be responsible for these phenotypic differences. A dynamic relationship appears to exist between the mosquito holobiont and WNV vector competence in *Cx. tarsalis*.

1365

ANOPHELES ARABIENSIS IS NOT SUCCESSFULLY CONTROLLED BY INDOOR RESIDUAL SPRAYING IN NORTHWEST TANZANIA: IMPLICATION FOR MALARIA VECTOR CONTROL IN THE AREA

Natacha Protopopoff[1], Jovin Kitau[2], Alexandra Wright[1], Philippa West[1], Franklin W. Mosha[2], William Kisinza[3], Immo Kleinschmidt[1], Mark Rowland[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Kilimanjaro Christian Medical University College, Moshi, United Republic of Tanzania, [3]National Institute for Medical Research, Muheza, United Republic of Tanzania

In East Africa scale up of Insecticide Treated Net (ITN) and Indoor Residual Spraying (IRS) has been associated with a drastic reduction in the abundance of *Anopheles gambiae* s.s. the primary vector in the area. This led to an apparent shift in An. gambiae s.l. sibling species ratio toward the more zoophilic and more exophilic *An. arabiensis* which is less likely be killed by IRS and ITN. The impact of IRS with bendiocarb on the relative abundance of *An. gambiae* s.s. and *An. arabiensis* was evaluated in North West part of Tanzania during a community randomised trial. Pre intervention, *An. arabiensis* represented 18.6% (95%CI: 13.9-24.6) and *An. gambiae* s.s. 81.4% (95%CI: 75.5-86.1) of the population of *An. gambiae* s.l. collected with indoor light traps, while *An. arabiensis* accounted for 3.8% and *An. gambiae* s.s. 96.2% of the population found resting indoor. Sporozoite rate was 1.4% (95%CI: 1.1-2.0) and only *An. gambiae* s.s. were found positive. After IRS, density of *An. gambiae* s.s was reduced by 75% (p=0.046) and *An. arabiensis* by 25% (p=0.745). In the IRS villages sporozoite rate in *An. gambiae* s.s. was 1.8% and 0% for An.arabiensis. There was a significant difference in the gambiae s.s./arabiensis species ratio with *An. arabiensis* constituting 11.3% the control arm alone compared to 26.1% in the IRS arm (OR: 2.8 (95%CI: 1.1-6.8) p=0.027). Indoor Residual Spraying was more effective in controlling *An. gambiae* than *An. arabiensis* in North West Tanzania. *An. arabiensis* in this area is a secondary vector and appeared to contribute little to malaria transmission. The focus of control should remain on *An. gambiae* s.s. the main vector in this area while more specific vector control tools for *An. arabiensis* could be investigated.

1366

TRANSCRIPTOMICS OF DIFFERENTIAL VECTOR COMPETENCE: WNV INFECTION IN TWO POPULATIONS OF *CULEX PIPIENS QUINQUEFASCIATUS* LINKED TO OVARY DEVELOPMENT

Chelsea T. Smartt, Dongyoung Shin

University of Florida, Vero Beach, FL, United States

Understanding mechanisms that contribute to viral dissemination in mosquito vectors will contribute to our ability to interfere with the transmission of viral pathogens that impact public health. The expression of genes in two *Culex pipiens quinquefasciatus* populations from Florida with known differences in vector competence to West Nile virus (WNV) were compared using high throughput sequencing. Results A total of 15,176 transcripts were combined for comparison of expression differences between the two populations and 118 transcripts were differentially expressed ($p<0.05$). The fold change in expression of the differentially expressed genes ranged from -7.5 - 6.13. The more competent population for WNV (Gainesville) over expressed 77 genes and down regulated 44 genes, compared with the less competent population for WNV (Vero Beach). Also, splicing analysis identified 3 transcripts with significantly different splice forms between the two populations. The functional analysis showed that the largest proportion of transcripts was included in the catalytic activity and transporter activity groups except for those in the unknown group. Interestingly, the up- regulated gene set contained most of the catalytic activity function and the down- regulated gene set had a notable proportion of transcripts with transporter activity function. Immune response category was shown in only the down regulated gene set, although those represent a relatively small portion of the function. Several different vitellogenin genes were expressed differentially. Based on the RNAseq data analysis, ovary development was compared across the populations and following WNV infection. There were significant differences among the compared groups. In conclusion, this study suggests that ovary development is related to vector competence in two Culex populations in Florida. Both populations control energy allocations to reproduction as a response to WNV. This result provides novel insight into the defense mechanism used by Culex spp. mosquitoes against WNV.

1367

POPULATION SUBDIVISION WITHIN *ANOPHELES GAMBIAE* MAY IMPACT MALARIA TRANSMISSION IN GUINEA BISSAU

José L. Vicente[1], Beniamino Caputo[2], Marco Pombi[2], Carla A. Sousa[3], João Dinis[4], Amabélia Rodrigues[4], David Weetman[5], Alessandra della Torre[2], **João Pinto**[1]

[1]Centro de Malária e outras Doenças Tropicais, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisbon, Portugal, [2]Istituto Pasteur-Fondazione Cenci-Bolognetti, Dipartimento di Sanità Pubblica e Malattie Infettive, Università di Roma "La Sapienza", Rome, Italy, [3]Unidade de Parasitologia e Microbiologia Médicas, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisbon, Portugal, [4]Instituto Nacional de Saúde Pública, Ministério da Saúde Pública, Bissau, Guinea-Bissau, [5]Department of Vector Biology, Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Anopheles coluzzii and *Anopheles gambiae*, formerly *Anopheles gambiae* s.s. M and S forms, are generally characterized by low levels of hybridization along most of their west African sympatric range. However, high levels of hybridization and genetic introgression have been detected in the western African limit of their distribution, particularly in Guinea Bissau. In this study, we have characterized levels of genetic differentiation within and between *A. coluzzii* (M-form) and *A. gambiae* (S-form) samples collected from 10 localities along an east-west transect in Guinea Bissau, during the rainy season of 2010. Samples were identified to species by IGS-rDNA and SINE200X markers, genotyped for 19 microsatellites and for the insecticide resistance associated ace-1 and kdr loci. In addition, ELISA was used to determine blood meal origin and to assess sporozoite

rates in selected localities. Microsatellite data showed that hybridization between *A. coluzzii* and *A. gambiae* occurs mainly in coastal areas, with hybrid rates up to 19.4%. Moreover, Bayesian clustering analysis revealed a subdivision within *A. gambiae* into east and west/coastal populations. These populations are geographically separated by a central region where *A. coluzzii* prevails. Genetic partitioning within *A. gambiae* was also evident from the distribution of ace-1 and kdr resistance-associated alleles, that reached frequencies of 3% and 83% in east localities but were absent from west/coastal sites. West/coastal *A. gambiae* presented human blood indexes (HBI) between 26.4% and 77.8% whereas in the east population HBI was 99.3%. Sporozoite rates between 3.7% (N= 54) and 7.2% (N= 69) were recorded in east populations of *A. gambiae* but no CSP-positive mosquitoes were detected in west/coastal populations (N= 196). The differences in anthropophily and sporozoite rates found between east and west/coastal populations suggest that the genetic partitioning within *A. gambiae* is likely to have an impact on malaria transmission in the country.

1368

ELIZABETHKINGIA ANOPHELIS: MOLECULAR MANIPULATION AND INTERACTIONS WITH MOSQUITO HOSTS

Shicheng Chen, Michael Bagdasarian, Edward Walker

Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI, United States

The microbiota of the mosquito gut can profoundly influence metabolism, fecundity, development and immunity of the mosquito host. Further, this microbiota may through natural processes or by paratransgenesis provide a promising method to constrain malaria parasite development. In this study, we used the bacterial commensal *Elizabethkingia anophelis* from *Anopheles gambiae* as a model to study its interaction with host mosquitoes. A genetic manipulation system involving plasmids, selectable markers, a reporter system, and transposons was newly developed for an *Elizabethkingia* strain isolated from our laboratory colony of *An*. *gambiae*. A replicable plasmid carrying the antibiotic resistance gene *ermF* was efficiently introduced into *Elizabethkingia* by conjugation from *E. coli*, resulting in erythromycin-resistant colonies. Plasmids from *Elizabethkingia* were successfully transferred to *Elizabethkingia* by electroporation, but transformation was at low frequency with the same plasmids and an *E. coli* donor, suggesting the presence of a restriction barrier. The transposon pMiniHimar-Em1 was conjugatively introduced into *Elizabethkingia* from *E. coli*. It transposed randomly, resulting in Em-resistant colonies; transposition efficiency was improved by modifying transposase promoter activity. A strong flavobacterial expression system based on promoter P*ompA* was engineered into pMiniHimar and adapted to *Elizabethkingia*. A GFP- and Nanoluc- tagged *E. anophelis* strain fed to larvae of *Anopheles gambiae* and *Aedes triseriatus* showed transtadial persistence and propagative growth in the *An. gambiae* gut environment but not in *Ae. triseriatus*, indicating that *Elizabethkingia* has a limited host range. Paratransgenesis potential of *Elizabethkingia anophelis* will be discussed.

1369

INFLUENCE OF DENGUE VIRAL TITER ON *AEDES AEGYPTI* BEHAVIORAL RESPONSE TO DEET

Victor A. Sugiharto[1], John P. Grieco[1], Michael J. Turell[2], V. Ann Stewart[1], Edgar D. Rowton[3], Michelle G. Colacicco-Mayhugh[4], Cara H. Olsen[1], Nicole L. Achee[5]

[1]Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [2]U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD, United States, [3]Walter Reed Army Institute of Research, Forest Glen, MD, United States, [4]Medical Education and Training Campus, Ft. Sam Houston, TX, United States, [5]University of Notre Dame, South Bend, IN, United States

Mosquito behaviors are heavily driven by odor cues within the surrounding environment. These cues are recognized by chemosensory receptors. Previous studies have shown that arboviral infections can alter mosquito behavior based on dysfunction of mosquito organs, particularly those of the nervous system.. Previously we have reported on the behavioral responses of DENV-1 infected mosquitoes exposed to DEET in the HITSS contact irritancy (CI) on 1, 4, 7, 10, 14, and 17 days post-injection (DPI). Here, we explore the association between dengue virus-1 (DENV-1) RNA copy in mosquito heads and their corresponding CI response in time series after infection. Viral RNA copy of individual head-preps of each DPI cohort are being used in reverse transcriptase RT-PCR to quantify viral RNA copy in both responders (irritated by DEET) and non-responders (no irritation upon exposure to DEET). Time to viral RNA copy plateau and, more importantly, differences in viral RNA copy between the responders and non-responders on any day post injection will be presented. Data will be used to determine the correlation between viral RNA titer with *Aedes aegypti* response against DEET. Findings will enhance our understanding of the potential attenuation in efficacy of chemical products designed to reduce the probability of human contact with infected vectors - a vital component for prevention of dengue virus transmission. Data collection will be completed by July 2014.

1370

EXPERIMENTAL EVIDENCE AND EMPIRICAL PROOF FOR CONTROL OF *PHLEBOTOMUS PAPATASI* SAND FLIES (DIPTERA: PSYCHODIDAE) USING A FEED-THROUGH LARVICIDAL RODENT BAIT WITH A BUILT-IN VALIDATION SCHEME

Thomas M. Mascari[1], Hanafi H. Hanafi[2], Souad Ouahabi[3], Btissam Ameur[4], Chafika Faraj[3], Peter J. Obenauer[2], Joseph W. Diclaro[2], Lane D. Foil[1]

[1]Louisiana State University, Baton Rouge, LA, United States, [2]U.S. Naval Medical Research Unit - 3, Cairo, Egypt, [3]Medical Entomology Laboratory, Rabat, Morocco, [4]Department of Epidemiology and Disease Control, Rabat, Morocco

Leishmaniasis is a neglected tropical disease for which very little can be done on an operational scale to reduce sand fly vector populations, parasite transmission, or the incidence of disease. Previously, control of sand fly larvae has not been an option because little is known about the larval ecology of most species, and because no reliable larval sampling method exists. Recently, however, stable isotope analysis of adult sand flies demonstrated that half of the sand fly larvae at a site in eastern Morocco developed to the adult stage on rodent feces. Previous lab studies have shown that rodent diets containing insecticides that pass into the feces effectively kill sand fly larvae. We report the results of a small-scale field trial on the use of feed-through larvicidal rodent baits to reduce sand fly populations. Half of the study sites received insecticidal rodent baits and half received untreated baits. Rather than solely measuring changes in the adult sand fly population, both the insecticidal and untreated rodent baits were co-formulated with a fluorescent tracer dye that passes into rodent feces, and marks both the sand fly larvae that feed on the feces and the subsequent adults. This tracer system provided a crucial entomological indicator: the proportion of adult sand flies that had fed as larvae on the feces of baited rodents. We observed significant reductions in the adult sand fly population and, through the use of the tracer, had empirical evidence for a causal link between the lower number of adult sand flies captured and the efficacy of the insecticidal rodent bait.

1371

A QUALITY MANAGEMENT SYSTEM FOR *ANOPHELES* INSECTARIES IN FDA-REGULATED STUDIES IN MALI

Mamadou Coulibaly[1], Joseph P. Shott[2], Brandyce St Laurent[3], Ibrahima Baber[1], Jennifer M. Anderson[3], Sara A. Healy[4], Yimin Wu[4], Issaka Sagara[1], Patrick E. Duffy[4], Robert W. Gwadz[3], Sekou F. Traore[1]

[1]Mali International Center for Excellence in Research, Malaria Research and Training Center, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali, [2]Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [3]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [4]Laboratory of Malaria Vaccinology and Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

Human feeding studies using endemic vectors for arthropod-borne diseases have become a cornerstone for evaluating novel interventions such as vaccines, drugs, and repellants. Potential barriers to evaluating these interventions in endemic settings include: ensuring the fitness and safety of the vectors, and competency of regulatory sciences by entomological staff, particularly when involving studies overseen by the U.S. Food and Drug Administration. As part of a Phase 1 trial of a *Plasmodium falciparum* transmission-blocking vaccine (Pfs25), we have created a Quality Management System for our *Anopheles* insectary in Mali. This QMS is based on the tenets of Good Laboratory Practices and Good Manufacturing Practices including: standard operating procedure improvement and harmonization, training and competency assessment, quality control & assurance, and rigorous documentation practices. Our efforts to ensure tightly regulated processes for the continuous production of high-quality, safe vectors for human studies may benefit other institutions involved in the entomological components of interventional studies.

1372

POPULATION DYNAMICS OF *AEDES AEGYPTI* AND *ALBOPICTUS* IN NEW ORLEANS, LA, 2009-2013

Justin K. Davis, Dawn M. Wesson

Tulane University, New Orleans, LA, United States

Given the recent identification of autochthonous cases of dengue virus (DENV) in Texas and Florida and the apparent introduction of chikungunya virus (CHIKV) to the western hemisphere, it is reasonable to suspect that the viruses will eventually reach endemicity in the New Orleans, LA area, where two competent vector species, *Ae. aegypti* and *albopictus*, have established abundant populations. To investigate their population dynamics, oviposition cups were used to solicit eggs, larvae, and pupae in various areas in New Orleans, LA between 2009 and 2013. Samples are related to remotely-sensed vegetation indices and to meteorological covariates by the gamma distributed lag model of Schmidt (1974). We conclude that the two species respond systematically but differently to environmental covariates, namely temperature and precipitation, and that different weather scenarios imply predictable differences in the risk to humans of these two viruses. A paradoxical result is identified, implying that oviposition cups have a methodological bias that must be understood in practice.

1373

OCCURRENCE OF PAROUS FEMALES IN COPULA IN NATURAL SWARMS OF *ANOPHELES GAMBIAE* S.L.: EVIDENCE FOR RE-MATING BETWEEN GONOTROPHIC CYCLES

Simon P. Sawadogo[1], Thierry Lefèvre[2], Abdoulaye Diabaté[1], Carlo Constantini[2], Rosemary S. Lees[3], Gabriella Gibson[4], Roch K. Dabiré[1]

[1]Institut de Recherche en Sciences de la Santé/Centre Muraz, Bobo-Dioulasso, Burkina Faso, [2]Laboratoire MIVEGEC (UMR CNRS 5290, IRD 224, UM1, UM2), Montpellier, France, [3]Polo d'Innovazione Genomica, Genetica e Biologia S.C.a.R.L, Perugia, Italy, [4]Natural Resources Institute/ University of Greenwich, London, United Kingdom

The mating behavior of mosquito disease vectors has important implications for the implementation of novel approaches to vector control, such as the sterile insect technique (SIT) and the release of genetically-modified mosquitoes. From 2006 to 2009, the natural swarming and mating behavior of *Anopheles gambiae* s.l. was investigated in two sites near Bobo-Dioulasso, Burkina Faso. The gonotrophic status, insemination rate and parity rate of indoor resting and swarming (pairing and single) female *An. gambiae* s.l. were determined. We report the presence of parous *An. gambiae* s.l. females mating in natural swarms. The parity rates of mating females, swarming single females and indoor-resting females were, respectively, 5.0% (30/606), 4.1% (21/517) and 16.9% (239/1416). Because females lay eggs only when inseminated, these observations indicate that re-mating can occur between gonotrophic cycles in *An. gambiae* s.l., the major vector of malaria in Sub-Saharan Africa.

1374

COMPARATIVE ABILITIES OF MICROFILAREMIC VERSUS NON-MICROFILAREMIC BIRDS TO INFECT *CULEX PIPIENS* MOSQUITOES WITH WEST NILE VIRUS

Jefferson A. Vaughan[1], Elizabeth S. Andrews[2], Juanita Hinson[2], Michael J. Turell[2]

[1]University of North Dakota, Grand Forks, ND, United States, [2]U.S. Army Medical Institute of Infectious Diseases, Frederick, MD, United States

Vertebrate reservoirs of arboviruses are often infected with microfilariae (MF). Previous laboratory studies have shown that MF can enhance the infectivity of arboviruses to mosquitoes. Soon after being ingested, MF penetrate the mosquito midgut. If the blood meal also contains virus (i.e., the vertebrate reservoir is dually-infected), penetrating MF may introduce virus into the hemocoel. This can transform otherwise virus-incompetent mosquito species into virus-competent species and simultaneously accelerate viral development, allowing mosquitoes to transmit virus sooner than normal. This phenomenon is termed microfilarial enhancement of arboviral transmission. Because the prevalence of MF is very high in many passerine populations in North America, we investigated if microfilarial enhancement by microfilaremic passerines could have facilitated the spread of West Nile virus (WNV) across the USA. To do this, we injected two groups of Common Grackles with WNV; one group possessed naturally-acquired infections of Chanderella quiscali MF (n=6), and one group did not have MF infections (n=4). Different batches of *Culex pipiens* mosquitoes were allowed to feed on these birds during the next two to three days and at various time points thereafter (days 3, 4, 5, 7 and 14), mosquitoes were tested by plaque assay to determine rates of WNV infection (i.e., increased vector competence) and dissemination (i.e., decreased extrinsic incubation period). At the time of this writing (April 2014), final data are still forthcoming and will be presented at the meeting.

1375

REDUCING *AEDES ALBOPICTUS* HUMAN LANDING RATES IN ITALY THROUGH INNOVATIVE MOSQUITO TRAPS

Scott W. Gordon[1], Christian Engelbrecht[2], Claudio Venturelli[3], Andreas Rose[1], Martin Geier[1]

[1]Biogents, Regensburg, Germany, [2]Institut für Zoologie, Regensburg, Germany, [3]Azienda Unità Sanitaria Locale, Cesena, Italy

Since its introduction and establishment in Italy during the early 90s, the Asian tiger mosquito has spread over large parts of Italy and other Mediterranean countries. *Aedes albopictus* is not only a cause of biting nuisance but also a competent vector for various arthropod-borne diseases. Conventional attempts to control *Ae. albopictus* include source reduction, larvicides and adulticides. Although efficient traps for *Ae. albopictus* exist and are used for population monitoring, their use as a control tool has not been extensively studied. In this study, we assessed the ability of BG-Sentinel mosquito traps to control local populations of *Ae. albopictus* over a 15-week period in Cesena, Italy. Six experimental sites were matched and paired for the criteria of urbanization level, surface vegetation and mosquito density. In each pair, one site was selected as an intervention site and treated with 7-8 traps. The other site was designated as a control site and did not receive traps. Trap density ranged from one trap per 150 m^2 to one per 300 m^2. Mosquito populations in both the intervention and in control areas were monitored weekly with human landing collections and ovitraps. Results from human landing collections indicated biting rates were reduced between 60 and 90% in the treatment areas compared to the untreated control sites. These results indicate that the sustained use of efficient mosquito traps can significantly reduce the nuisance caused by *Ae. albopictus* in residential areas.

1376

DATA-DRIVEN MODELING FOR RECEPTIVITY AND SPREAD OF THE HIGHLY INVASIVE MOSQUITO, *AEDES ALBOPICTUS*

Christopher M. Barker[1], Diego Montecino[1], Susanne Kluh[2], Kenn Fujioka[3], Tanya Posey[2], Brian Sorvillo[3], Wakoli Wekesa[3]

[1]University of California Davis, CA, United States, [2]Greater Los Angeles County Vector Control District, Santa Fe Springs, CA, United States, [3]San Gabriel Valley Mosquito and Vector Control District, West Covina, CA, United States

The mosquito, *Aedes albopictus*, is among the world's most invasive species. Its spread has been facilitated by rapid global transport of cargo and potentially by climate warming, and it is now established on every continent except Antarctica. This species represents a "triple threat" to human health, being a day-biting pest, a competent vector of globally important dengue and chikungunya viruses, and a potential bridge vector of several zoonotic arboviruses. As a result of its importance, the biology of *Ae. albopictus* is also well-studied, but the fine-scale processes by which it becomes established in a given location are poorly understood because even intensive surveillance systems yield limited information during the early phase of invasions when densities are low, and detection often occurs after populations are relatively widespread. Fine-scale spatial models for mosquito dynamics and movement offer a way forward, marrying our understanding of *Ae. albopictus* biology with surveillance paradigms and detailed data on the real landscapes where invasions occur. Here, we consider the ongoing invasion and establishment of *Ae. albopictus* in Los Angeles since late 2011. We use hierarchical modeling with remote sensing and surveillance data from the study area to account for heterogeneities in household-level receptivity, then we model the stochastic dynamics of *Ae. albopictus* on this landscape using the suitability surface and a temperature-dependent, dynamical model for reproduction and spread. We found the probability of establishment to be much greater for introductions of eggs in containers compared to single adult females that might arrive by automobile. We also show that the rate of spread was strongly seasonal and greatest during late spring and summer, and the ability to contain the mosquitoes' spread diminished rapidly with increasing delays to detection, regardless of the control methods used.

1377

VILLAGE EDGE ASSOCIATION, DIEL FLIGHT ACTIVITY AND HOST SELECTION PATTERNS OF MALARIA VECTORS IN VILLAGES OF MADANG PROVINCE, PAPUA NEW GUINEA

John B. Keven[1], Edward D. Walker[2], Lisa Reimer[3], Peter M. Siba[1], James W. Kazura[4], Peter A. Zimmerman[4]

[1]Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea, [2]Michigan State University, East Lansing, MI, United States, [3]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [4]Case Western Reserve University, Cleveland, OH, United States

The diel flight and host seeking behavior of females of 5 species of *Anopheles* mosquitoes was studied in 4 malaria-endemic villages of Madang Province, Papua New Guinea, using a vertical barrier screen sampling system, during May to August, 2012. The screen consisted of shade cloth configured to posts and erected vertically to a height of 2.5 meters. It captured both host seeking and blood fed individuals throughout the night. More non-blood fed females were captured on the side of the screen facing the bush, earlier in the evening, whereas more blood fed females were captured on the village side of the screen later in the evening to early morning. These results suggest commuter behavior of host seeking females from outside to inside the village nightly, followed by village exiting behavior back to the surrounding bush. Host identification of blood meals by sequencing of the mitochondrial cytochrome B gene revealed that humans and domestic pigs were the most common and often only hosts, even though other potential vertebrate hosts were present in abundance. *An. punctulatus* and *An. koliensis* were highly anthropophagous, *An. farauti* s.s, *An. longirostris*, and *An. farauti* (species 4) relatively less so, whilst *An. bancrofti* fed mostly on pigs. The implications of these findings for malaria transmission are discussed with reference to the human blood index.

1378

PRE-CLINICAL EVALUATION OF A COMBINED LIVE ATTENUATED (LAV) AND SUBUNIT (DEN-80E) PRIME-BOOST VACCINE APPROACH AGAINST DENGUE

Danilo R. Casimiro[1], Beth-Ann Coller[1], Steve Meschino[1], Elizabeth Thoryk[1], Ramesh Chintala[1], Govindarajan Dhanasekaran[1], Stephen Whitehead[2], Andrew J. Bett[1]

[1]Merck Research Labs, West Point, PA, United States, [2]Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

Merck & Co is developing a tetravalent recombinant subunit vaccine for dengue. The current vaccine candidate (referred to as V180) consists of four truncated, soluble dengue envelope protein (DEN-80E from DENV1-4) produced in Drosophila S2 cells and administered with a saponin-based adjuvant, ISCOMATRIX™ adjuvant. In previous non-human primate studies V180 was able to (a) induce a balanced immune response across all 4 types and (b) protect against virus challenge in rhesus macaques. It is currently the subject of a Phase I trial in flavivirus naïve subjects. While the inclusion of a novel adjuvant in V180 appears necessary to induce a rapid robust response, it may also complicate the development path. In contrast, live attenuated viral (LAV) candidates typically have good immunogenicity, memory/durability, and favorable CoGs but may be complicated by interference, under/over-attenuation, and/or extended dosing schedules. The recently reported poor efficacy of the chimeric dengue vaccine against DENV2 in a Phase II trial may also suggest that the induced titers may not be sufficient to provide protective efficacy in the field. For this reason, we have conducted rhesus macaque studies in which the tetravalent DEN-80E vaccine (with or without the use of ISCOMATRIX™) is combined with a tetravalent LAV in a heterologous prime-boost immunization regimen.

The objective is to optimize the neutralizing titers across all 4 types for both magnitude and longevity. Immunological data on the heterologous and homologous prime-boost vaccinated monkeys will be presented. The combined use of live/non-live vaccine immunogens has the potential of being an effective vaccine approach against multiple dengue types.

1379

COMPARISON OF PASSIVE AND SENTINEL-ENHANCED DENGUE SURVEILLANCE SYSTEMS IN PUERTO RICO

David Noyd[1], Olga Lorenzi[1], Aidsa Rivera[1], Janice Perez Padilla[1], Luisa Alvarado[2], Kay M. Tomashek[1], **Tyler M. Sharp**[1]

[1]Centers for Disease Control and Prevention, San Juan, PR, United States, [2]San Lucas Episcopal Hospital, Ponce, PR, United States

Dengue represents an increasingly important global and public health challenge; however, current passive surveillance systems underestimate the true burden of disease. In 2009, the World Health Organization urged countries to implement sentinel-enhanced surveillance to characterize the epidemiology of dengue to better evaluate new prevention methods. To estimate underreporting of dengue via passive surveillance in Puerto Rico, we analyzed the epidemiologic trends of suspected dengue cases reported to the long-running island-wide passive dengue surveillance system (PDSS) and compared them to those obtained from cases identified by a hospital-based Sentinel Enhanced Dengue Surveillance System (SEDSS). Dengue diagnostic testing for both PDSS and SEDSS includes RT-PCR to detect dengue virus (DENV) nucleic acid and ELISA to detect anti-DENV IgM antibody. Analyzed data were collected from PDSS and SEDSS in the Ponce health region between May 7, 2012 and May 6, 2013, the first year of operation of SEDSS. Of 3,483 suspected dengue cases reported to PDSS and 2,027 cases identified by SEDSS, 1,444 (41.5%) and 621 (30.6%) were laboratory-positive dengue cases, respectively. Compared to dengue cases reported to PDSS, those identified by SEDSS were younger (25 years vs. 19 years; $p < 0.0001$), presented for care earlier after illness onset (3 days vs. 4 days; $p < 0.0001$), were hospitalized less frequently (46% vs. 64%; $p < 0.0001$), and demonstrated higher completion of demographic and clinical variables for the case investigation form (61% vs. 27%; $p < 0.0001$). There were no significant differences in other demographic variables or DENV type distribution between SEDSS and PDSS. This evaluation demonstrated that SEDSS provides more robust clinical information and more accurately identifies non-hospitalized patients, though it may bias toward younger individuals. Enhanced dengue surveillance should be implemented in other locations of the world to complement existing passive surveillance systems to better understand the epidemiology and burden of dengue.

1380

WHAT PROPORTION OF DENGUE VIRUS INFECTIONS RESULT IN NO APPARENT DISEASE?

Hannah E. Clapham[1], Derek A. Cummings[1], Michael A. Johansson[2]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Division of Vector Borne Diseases, Centers for Disease Control and Prevention, San Juan, PR, United States

The concept of the dengue iceberg is a well-known one; a large proportion of dengue virus infections result in minimal or no symptoms and are thus never reported to surveillance systems. However, there is no consensus on what this proportion is and estimates in the literature vary substantially. We define infections as either apparent or inapparent. Though the definition of an apparent infection may vary depending on the study methodology, this is generally a dengue virus infection that results in overt illness, symptoms or healthcare seeking. An inapparent infection is one in which individuals have a serological response consistent with infection, but no accompanying illness (as defined above). Estimates in the literature of the proportions of dengue virus-infected people who experience apparent or inapparent infections vary widely. Though some of this variation may be due to differential definitions, there is some evidence that these proportions depend not only on whether the infection is a first, second or post-second infection, but also on infecting serotype (and in some cases genotype) and on the age of the infected individual. Combining published data from dengue cohort studies and from outbreak situations with serological data from multiple settings over multiple years, with consideration of the definitions used in each study, we aim to estimate the proportion of dengue virus infections that are apparent and inapparent. We also aim to estimate the influence of immune history, infecting serotype and age on these proportions. By combining datasets, the similarities and differences between these settings provide increased information about the effect of each of these factors. A Bayesian framework is used, thereby allowing the inclusion of uncertainty in the data and our resulting estimates. Better estimates of the proportions of dengue virus infections that are inapparent will be of use for understanding transmission of dengue viruses in multiple settings. For example, inapparent infections may be contributing to transmission. In addition, inapparent primary infections, though not producing apparent infection and thus not contributing to disease burden, leave individuals primed for secondary infections, so will be important for understanding the future disease burden in a population.

1381

CLINICAL COURSE AND OUTCOME OF DENGUE INFECTION DURING PREGNANCY

Ananda D. Wijewickrama[1], Gaveshika Abeyratne[1], Damayanthi Idampitiya[1], Priyankara Jayawardena[2]

[1]Infectious Diseases Hospital, Angoda, Sri Lanka, [2]Castle Street Hospital for Women, Colombo, Sri Lanka

Dengue, a major mosquito borne infection in the tropics, is hyper-endemic in Sri Lanka since 2009 with an annual incidence of more than 25,000 cases. The maximum rate of infection is seen in the 20-39 age category, making the pregnant population a vulnerable category.A retrospective observational study was conducted on all pregnant patients with Dengue admitted during 2013 to two major hospitals in Colombo, Sri Lanka. Data including clinical & laboratory parameters, interventions made, and complications were documented for analysis.Dengue infection was confirmed in 58 patients admitted. Mean age was 28.45(SD: 5.573) yrs. 55.2% had Dengue fever (DF) while 44.8% had Dengue Haemorrhagic fever (DHF). 19%, 46.6% and 34.5% were in the first, second and third trimester respectively. 82.8% had Rh positive blood groups, with 27.6% B positive, 25.9% O positive, 20.7% A positive and 8.6% AB positive while in 3.8% the blood group was not known. All had fever and 86.2% had myalgia. Hepatic tenderness, persistent vomiting and postural dizziness were seen most commonly with DHF (81.8%, 100% & 70% respectively). Mean day of entry into critical phase was 4.5 (SD: 0.990) day of the illness. The mean lowest platelet count in DF was 90.94 while in DHF was 37.81 ($p<0.000$), which was observed on a mean day of 5.25 in DF & 6.04 in DHF ($p< 0.03$). Most of the DF patients (31.2%) had highest AST levels in the 32-100 range while most of the DHF patients (34.6%) had levels in the 501-1000 range. Most of the DF patients (34.4%) had normal ALT level whereas most of the DHF patients (38.5%) had highest ALT in the 101-300 range. 3.1% of DF and 7.7% of DHF patients had fetal distress while 3.1% of DF and 3.8% of DHF patients had intrauterine death (IUD). 52% of all patients needed HDU/ICU care. All the patients recovered completely. This study shows incidence of DHF is higher in pregnancy than in the normal population. High numbers of patients with Rh positive blood groups were among pregnant Dengue patients. Some parameters like low platelet count, high AST and ALT were significantly high in DHF, indicating these can be used to identify high risk groups for developing DHF. Careful management assures full recovery of mothers, however, adverse outcome on the fetus remains high.

1382

USE OF A HOUSEHOLD SEROSURVEY TO ESTIMATE THE MAGNITUDE OF A DENGUE OUTBREAK IN MOMBASA, KENYA, 2013

Esther M. Ellis[1], John Neatherlin[2], Abdinoor Mohamed[2], Barry Fields[2], Daniel Ondari[2], Shem Patta[3], Mark Delorey[4], Brenda Torres-Velasquez[1], Nina Marano[2], Lilian Waiboci[2], Melvin Ochieng[2], Stella Gikunju[2], Harold Margolis[1], Joel Montgomery[2], Kay M. Tomashek[1]

[1]Centers for Disease Control and Prevention, San Juan, PR, United States, [2]Kenya Medical Research Institute/Centers for Disease Control and Prevention, Nairobi, Kenya, [3]Ministry of Health, Mombasa, Kenya, [4]Centers for Disease Control and Prevention, Fort Collins, CO, United States

Dengue is endemic in Africa where an estimated 64 million dengue virus (DENV) infections occurred in 2010. Few outbreaks have been reported from East Africa since dengue was first detected in the late 1800s, and the geographic distribution of infections is uncertain. In February 2013, several individuals with dengue-like illness and negative malaria blood smears were identified in Mombasa, Kenya. Serum samples from initial cases confirmed dengue, and an investigation was conducted to determine the incidence of DENV infection in Mombasa. A stratified multistage sample of households was selected from the Tudor community of Mombasa where the incidence of reported dengue was high. Household residents provided serum specimens and information on medical and travel history. Serum was tested for DENV nucleic acid by RT-PCR, NS1 ELISA (i.e., current DENV infection), and anti-DENV IgM antibody by ELISA (i.e., recent DENV infection). Design-based estimates incorporated probabilities for selection of households and used a finite population correction factor. Of 1,502 participants living in 701 households, 207 (14%) had evidence of current (n = 103) or recent (n = 104) DENV infection, with a design-based estimate of 13% (95% CI: 10-16). DENV-1 and -2 were detected equally. Of the 207 participants with evidence of DENV infection, 91 (44%) reported fever in the past month; three (1%) were hospitalized; and two (1%) had bleeding manifestations. Reporting a fever in the past 30 days was significantly associated with DENV infection (OR=2.8; CI 1.9-4.2). Reporting open windows at nighttime was a risk factor for infection (OR=2.3; CI 1.1-4.8). Daily use of mosquito repellent daily was protective from infection (OR=9.1; CI 3.7-20.0). This investigation revealed a high burden of dengue in this part of East Africa. Behavioral strategies to avoid mosquito bites should be advocated for individuals to avoid DENV infection. Surveillance for and clinical and public awareness of dengue should be improved in East Africa to reduce the morbidity and mortality due to this disease.

1383

DEVELOPMENT OF A NS1AG-ELISA FOR THE DETECTION OF ALL DENGUE 1 TO 4 TYPES

Katia Souza[1], Gérman Anez[2], Andréa Teixeira - Carvalho[3], Luz Espina Gomes[4], Caren Chancey[2], Andriyan Grinev[2], Daniel Heisey[2], Erna Kroon[5], Maria Rios[2]

[1]Departamento de Bioquímica e Imunologia, Belo Horizonte, Brazil, [2]Food and Drug Administration, Bethesda, MD, United States, [3]Centro de Pesquisa Rene Rachou, Belo Horizonte, Brazil, [4]Seccion de Virologia, Instituto de Investigaciones Clinicas, Maracaibo, Brazil, [5]Laboratório de Vírus Departamento de Microbiologia, Belo Horizonte, Brazil

Dengue is the most important arthropod-borne viral disease in tropical and subtropical areas of the world. It is a public health concern in the Americas, including the US, because it is endemic in Puerto Rico and caused outbreaks in Florida, Texas, Hawaii, and in other US islands. The infection is mainly transmitted by the mosquito *Aedes aegypti*. Dengue diagnosis is commonly made using MAC-ELISA which indirectly detects specific antibodies to the E protein of the Dengue virus (DENV). Direct detection by virus isolation in culture and reverse transcriptase-polymerase chain reaction (RT-PCR) is used less frequently due to time, limited availability and high cost. An attractive alternative to culture and molecular tests is the detection of NS1 in the sera of patients during the acute phase of dengue. This viral protein is produced and secreted by infected human cells in the early stages of infection. Unfortunately, NS1 assays currently available have low sensitivity and specificity, and have been reported to miss DENV-4. In order to address this issue we developed an ELISA-based NS1 antigen assay (NS1Ag-ELISA) that uses two monoclonal antibodies, selected among 10 commercially available, that recognizes all DENV1-4 NS1 proteins. Initially, we were tested: (a) 130 specimens positive for DENV RNA and/or IgM (29 DENV-1, 4 DENV-2, 7 DENV-3, 16 DENV-4 and 54 not typed), all produced positive results on the NS1Ag-ELISA. (b) 75 DENV-negative specimens (19 West Nile Virus (WNV) positive, 6 Yellow Fever Virus (YFV) positive and 50 negative). 66 tested negative while 9 (8 WNV-positive and 1 YFV-positive), tested negative for DENV by RT-PCR but produced false positive results in our assay, possibly due to cross-reactivity between Flavivirus NS1 under the current assay conditions. We are modifying the assay to enhance DENV NS1 specificity and reduce cross reactivity. We expect to have in a few months a robust diagnostic test for the acute phase of dengue infection that may help early identification of serious dengue disease and facilitate critical care.

1384

SPATIAL CLUSTERING OF DENGUE AT THE HOUSEHOLD LEVEL IN A HIGHLY URBAN SETTING

Katherine Anders[1], Tran Van Ngoc[2], Nguyen V. Chau[2], Nguyen Dac Tho[3], Cameron Simmons[1]

[1]Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam, [2]Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam, [3]Preventive Medicine Centre, Ho Chi Minh City, Vietnam

In the absence of a vaccine or therapeutic, the only strategy currently available for dengue prevention is control of the mosquito vector *Aedes aegypti*. In many endemic countries, including Vietnam, this is pursued primarily by responsive insecticide spraying around homes of reported cases. Central to this approach is the assumption that most DENV infections are acquired in the home. Evidence of highly focal DENV transmission at household level has been demonstrated in a rural village setting in Thailand, but it is unclear whether this assumption is valid in highly urban, mobile populations. We conducted a community-based study to investigate clustering of dengue risk around households in Ho Chi Minh City, Vietnam. We enrolled clusters of 25-35 household members and neighbours living within 25 metres of an index case with clinically suspected dengue. Laboratory diagnosis of the index cases allowed us retrospectively to classify them as confirmed dengue cases (n=52) or non-dengue controls (n=19), and to calculate the relative risk of 1) incident DENV infection during a two-week follow up period and 2) recent DENV infection at baseline in case clusters compared to controls (representing background risk). There was no difference in the risk of incident DENV infection between case and control clusters (82 in 1341 participants (6.1%) vs 31/569 (5.4%), respectively). However participants in case clusters were significantly more likely to have had a recent DENV infection at baseline than those in control clusters (OR = 2.3; 95%CI 1.2-4.7). The prevalence of DENV-infected *Ae. aegypti* collected from index houses was low overall (1%), with no difference between cases and controls, however case houses were significantly more likely to have high adult vector densities than controls. Our findings show that although there was clustering of recent DENV infections around households, there was no excess of incident infections in the two weeks following index case detection; this suggests that responsive vector control activities in this window are unlikely to have a large impact on DENV transmission in this setting.

1384

SPATIAL CLUSTERING OF DENGUE AT THE HOUSEHOLD LEVEL IN A HIGHLY URBAN SETTING

Katherine Anders[1], Tran Van Ngoc[2], Nguyen V. Chau[2], Nguyen Dac Tho[3], Cameron Simmons[1]

[1]Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam, [2]Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam, [3]Preventive Medicine Centre, Ho Chi Minh City, Vietnam

In the absence of a vaccine or therapeutic, the only strategy currently available for dengue prevention is control of the mosquito vector *Aedes aegypti*. In many endemic countries, including Vietnam, this is pursued primarily by responsive insecticide spraying around homes of reported cases. Central to this approach is the assumption that most DENV infections are acquired in the home. Evidence of highly focal DENV transmission at household level has been demonstrated in a rural village setting in Thailand, but it is unclear whether this assumption is valid in highly urban, mobile populations. We conducted a community-based study to investigate clustering of dengue risk around households in Ho Chi Minh City, Vietnam. We enrolled clusters of 25-35 household members and neighbours living within 25 metres of an index case with clinically suspected dengue. Laboratory diagnosis of the index cases allowed us retrospectively to classify them as confirmed dengue cases (n=52) or non-dengue controls (n=19), and to calculate the relative risk of 1) incident DENV infection during a two-week follow up period and 2) recent DENV infection at baseline in case clusters compared to controls (representing background risk). There was no difference in the risk of incident DENV infection between case and control clusters (82 in 1341 participants (6.1%) vs 31/569 (5.4%), respectively). However participants in case clusters were significantly more likely to have had a recent DENV infection at baseline than those in control clusters (OR = 2.3; 95%CI 1.2-4.7). The prevalence of DENV-infected *Ae. aegypti* collected from index houses was low overall (1%), with no difference between cases and controls, however case houses were significantly more likely to have high adult vector densities than controls. Our findings show that although there was clustering of recent DENV infections around households, there was no excess of incident infections in the two weeks following index case detection; this suggests that responsive vector control activities in this window are unlikely to have a large impact on DENV transmission in this setting.

1385

EPITOPE PRESERVATION, IMMUNOGENICITY AND PROTECTION OF INACTIVATED DENGUE VIRUS ANTIGENS FORMULATED WITH A NOVEL BIOLOGICAL ADJUVANT IN RHESUS MACAQUES

Laura J. White[1], Wahala Wahala[1], John Gibbs[1], Melissa Mattocks[1], Idia Rodrigez[2], Melween Martinez[2], Teresa Santiago[2], Petraleigh Maldonado[2], Robert Johnston[1], Aravinda de Silva[3], Carlos Sariol[2]

[1]Global Vaccines Inc., Research Triangle Park, NC, United States, [2]University of Puerto Rico, San Juan, PR, United States, [3]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

Dengue viruses (DENV1-4) are considered the most important emerging, human arboviruses with worldwide distribution in the tropics, yet there are no licensed antiviral therapies or vaccines available. Although there are live attenuated virus vaccine candidates in clinical trials, there is an urgent need to accelerate the development of second-generation vaccine strategies. We developed a dengue vaccine based on a purified, inactivated virion (iDV) mixed with a novel alphavirus adjuvant (GVI3000/3A). The GVI adjuvants are disarmed viruses that derive their activity from the replication of a truncated alphavirus RNA. *In vivo*, the GVI adjuvants target DC in the DLN and mimic the earliest stages of a viral infection. The antigenic integrity of purified dengue virus antigens (iDV) was determined after inactivation by different protocols. A panel of mouse and human MAbs were used as probes to confirm the preservation of conformational epitopes in different domains of E protein, including recently characterized serotype specific, strongly neutralizing human MAbs that map to epitopes only preserved in the quaternary structure of the virion. Safety, immunogenicity and protective efficacy of GVI-adjuvanted iDV were determined in rhesus macaques, comparing 3 adjuvant doses and 2 adjuvant modalities. A tetravalent iDV mixture formulated with a GVI adjuvant demonstrated 1) significant increases in neutralizing antibody titers, 2) protection from viremia, and 3) no adverse events in any of the vaccinated animals. These results support the advancement of this new dengue vaccine candidate toward clinical trials in humans.

1386

THE DETECTION OF ANTI-DENGUE VIRUS IGM IN URINE AS A PUTATIVE MARKER FOR SEVERE DISEASE

Elba V. Caraballo, Katie Poole-Smith, Kay M. Tomashek, Brenda Torres, Elizabeth Hunsperger

Centers for Disease Control and Prevention, Division of Vector-Borne Diseases, Dengue Branch, San Juan, PR, United States

Dengue is globally the most important arbovirus disease with an estimated 300 million dengue virus (DENV) infections however, only 100 million dengue cases are reported per year. It is estimated that 5-10% of cases result in severe dengue, which may include glomerular changes associated with renal dysfunction. We looked for the presence of anti-DENV IgM antibodies in urine as an indicator for severe dengue among patients identified with acute febrile illness in our Sentinel Enhanced Dengue Surveillance System (SEDDS) site in Ponce, Puerto Rico. Between May 2012-March 2013, 1560 patients with fever or history of fever for ≤7 days were enrolled, a past medical history of chronic illnesses was obtained, and they were followed through their febrile illness. Serum and urine specimens were collected during the acute (days post onset of fever (dpo)=0-5) and convalescent phase (dpo=6-14) of their illnessAcute serum was tested for DENV RNA by RT-PCR . All urine specimens were tested for anti-DENV IgM. The results from the urine anti-DENV IgM were compared to the results in serum to determine sensitivity and specificity. The sensitivity of urine anti-IgM was 37% and specificity was 98% compared to serum. When compared to serum RT-PCR results, the sensitivity of IgM in urine was 24% and the specificity was 93%. To determine if IgM in urine might be an early indicator of disease severity, we compared this result to patient hospitalization; hospitalization being used as a surrogate for disease severity. Hospitalized dengue patients were 3.2 times more likely to test positive for IgM in urine than IgM negative (OR = 3.2 95CI 4.9-2.2).There was no correlation between the presence of IgM in urine with sex, age or pre-existing chronic diseases such as diabetes, high blood pressure, or anemia. While detection of anti-DENV IgM in urine lacked adequate diagnostic sensitivity when compared to serum, its presence may be a marker for hospitalization or disease severity.

1387

DEVELOPMENT OF DENGUE VIRUS PRM-REACTIVE ANTIBODIES AS TOOLS FOR MEASURING THE VIRION MATURATION STATE OF INFECTIOUS VIRIONS

Swati Mukherjee[1], Soila Sukupolvi-Petty[2], Aihua Zheng[3], Margaret Kielian[3], Michael S. Diamond[2], Theodore C. Pierson[1]

[1]National Institutes of Health, Bethesda, MD, United States, [2]Departments of Medicine, Molecular Microbiology, Pathology, Washington University School of Medicine, St. Louis, MO, United States, [3]Department of Cell Biology, Albert Einstein College of Medicine, Bronx, NY, United States

Newly formed dengue viruses (DENV) incorporate envelope (E) proteins in complex with the viral structural protein premembrane (prM) as heterotrimeric spikes. During egress from infected cells, prM is cleaved to pr and M protein by host furin-like proteases to produce infectious virions. However, we and others have shown that this process is inefficient and leads to the release of partially mature DENV that retain non-cleaved

prM and have significantly different functional properties. The extent of prM cleavage required for production of an infectious virus is unknown. In this study, we characterized a panel of murine monoclonal antibodies (mAbs) that bind prM produced by immunization with recombinant pr protein. prM-reactive mAbs were extensively cross-reactive and shown to be capable of enhancing DENV infection of Fc-receptor-expressing cells. Several prM-reactive antibodies displayed a significant capacity to neutralize infection, although this pattern was cell type-dependent. Examination of neutralization dose-response curves on Raji cells expressing DC-SIGNR revealed the presence of a fraction of virions resistant to neutralization; the size of this population could be varied by altering the efficiency of the virion maturation process. We conclude that in this context, viruses resistant to neutralization are those that display prM epitopes with a stoichiometry insufficient to satisfy the threshold requirements for neutralization. We demonstrate the potential for prM-reactive antibodies as a sensitive functional probe of the maturation state of DENV released from cells, providing a method to deconstruct the structural heterogeneity of DENV produced under a variety of conditions.

1388

COSTS OF DENGUE FEVER TO THE HEALTH SYSTEM AND INDIVIDUALS IN COLOMBIA IN 2010 TO 2012

Raul Castro Rodriguez[1], Katia Galera[1], Juan Guillermo López Yescas[2], Jorge Rueda[1]

[1]Universidad de los Andes, Bogota, Colombia, [2]Sanofi Pasteur, Mexico City, Mexico

Dengue fever is an important health issue in Colombia but detailed direct information on economic costs is lacking. We estimated the average cost per case of ambulatory dengue fever (aDF), hospitalized dengue fever (hospDF) and dengue hemorrhagic fever (DHF) over the period. Tallied costs included direct and indirect medical costs, as well as non-medical costs to the healthcare system, and indirect costs to patients, using information from official databases and an extensive population-based face-to-face survey of 1,089 households with recent dengue fever patients. In 2010, the mean direct medical cost per case for the healthcare system of aDF, hospDF, and DHF were, respectively $52.8USD, $235.8, and $1,512.2. To the individuals, the mean direct non-medical costs ($29.7, $46.7 and $62.6, respectively) greater than the mean household direct medical costs ($13.3, $348 and $57.3, respectively). The average cost to the healthcare system of a case of ambulatory dengue fever in the epidemic year of 2010 was 57% that in 2011.Our results highlight the high economic burden of the disease and could be useful for assigning limited health resources..

1389

INNATE IMMUNITY AND TRANSCRIPTOME PROFILING AFTER ADMINISTRATION OF TAKEDA'S LIVE ATTENUATED DENGUE VACCINE CANDIDATE IN FLAVIVIRUS-NAÏVE HUMAN VOLUNTEERS: ASSOCIATION OF GENE EXPRESSION WITH DEVELOPMENT OF NEUTRALIZING ANTIBODY RESPONSES TO DENV

Sarah George[1], Jorge Osorio[2], Dan T. Stinchcomb[2], Aurelia Haller[2], Ellis Reinherz[3], Vladimir Brusic[3]

[1]St. Louis University, St. Louis, MO, United States, [2]Takeda Vaccines, Inc., Madison, WI, United States, [3]Dana Farber Cancer Institute, Boston, MA, United States

We conducted a phase I, randomized, double-blind dose-escalation study of two different formulations of Takeda's live attenuated dengue vaccine candidate in 72 healthy flavivirus-naïve adults at the Saint Louis University VTEU (NCT01110551). Volunteers received 2 doses of a live attenuated tetravalent dengue vaccine candidate 90 days apart. Samples of whole blood were collected on days 0, 2, 4, 7, and 11 after vaccination to assess early responses to the vaccine by transcriptome analysis. Total RNA was isolated from whole blood and T7 transcribed-linear RNA was amplified and hybridized to Illumina Human HT12 v 4 microarrays. These microarrays detect all mRNAs expressed from the human genome. RNA expression data was exported and analyzed by genesets using the canonical pathways stored within the MSigDB database. No significant changes in geneset expression were identified that correlated with: 1) route of vaccine administration (SC vs. ID), 2) viremia after vaccination, or 3) neutralizing antibody titer (above or below the group mean on day 120 after vaccination). However, there were significant differences in geneset expression in subjects who developed a tetravalent neutralizing antibody response to DENV vs. subjects who had a mono/bivalent response. Subjects with a tetravalent response had at least a 1.5-fold increase in expression of genesets involved in integrin signaling, the complement pathway, interferon signaling, cytokine expression, and innate immune responses. In summary, increased expression of genesets which mediate the innate immune response and translation to adaptive immunity was significantly correlated with a tetravalent neutralizing antibody response to Takeda's live attenuated dengue vaccine candidate.

1390

IMPORTATION FOLLOWED BY LOCAL TRANSMISSION OF TWO LINEAGES OF DENGUE VIRUS TYPE 1 IN THE UNITED STATES: 2013

Gilberto A. Santiago[1], Dana Thomas[1], Brian Smith[2], Danielle Stanek[3], Ana M. Rivas[4], Carina Blackmore[3], Linda Gaul[2], Kay Tomashek[1], Jorge L. Munoz-Jordan[1]

[1]Centers for Disease Control and Prevention, San Juan, PR, United States, [2]Department of State Health Services, Texas, Harlingen, TX, United States, [3]Florida Department of Health, Tallahassee, FL, United States, [4]Universidad Autonoma de Monterrey, Monterrey, Mexico

Dengue, one of the most important arthropod-borne tropical diseases globally, is a significant public health concern affecting an estimated 100 million people in 2010. Dengue is caused by any of the four genetically related dengue viruses (DENV-1-4) that are maintained by transmission in large urban areas by *Aedes* mosquitoes. The proliferation of urban areas, frequent international travel and climatic changes, have been proposed to contribute to the increased dissemination of DENV-1-4. We have previously reported local transmission of a monophyletic lineage of DENV-1 in Key West, Florida in 2011-2012. In 2013, DENV-1 was identified in febrile patients residing in two Texas counties, Cameron and Hidalgo, adjacent to the border with Mexico. These patients reported no recent travel history. DENV-1 was also identified in febrile patients with no recent travel history residing in two Florida counties, Martin and St. Lucie. Identification of patients with laboratory-confirmed DENV-1 continued for time periods of 4-6 months in both locations. In this study we have conducted an in-depth envelope gene sequence analysis to characterize the emergence of DENV-1 in Texas and Florida and their relatedness with viruses from Key West, Mexico, Central America and the Caribbean. All sequences grouped within the American-African genotype of DENV-1. Bayesian phylogenetic analyses show a strong association of Texas and Northern Mexico DENV-1 with viruses from Central America, with the Texas isolates forming a monophyletic group. In contrast, the Florida isolates formed two independent subgroups: the previously reported Key West virus of Central American origin and the Martin-St. Lucie virus of Caribbean origin. The monophyletic characteristic of these lineages supports local transmission of DENV-1, and that conditions are suitable to sustain transmission with the potential to cause outbreaks.

1391

DENGUE QUASISPECIES COMPLEXITY ANALYSIS FOR MOSQUITO AND HUMAN SAMPLES FROM KAMPHAENG PHET, THAILAND: CLONING AND IMPLICATIONS FOR HIGH THROUGHPUT SEQUENCING METHODS

Melanie C. Melendrez[1], Piyawan Chinnawirotpisan[2], Alongkot Ponlawat[2], Chonticha Klungthong[2], Tao Li[1], Stephen J. Thomas[1], Robert V. Gibbons[2], Alan Rothman[3], Timothy P. Endy[4], In-Kyu Yoon[2], Thomas W. Scott[5], Jason H. Richardson[1], Richard G. Jarman[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [3]University of Rhode Island, Providence, RI, United States, [4]SUNY Upstate Medical University, Syracuse, NY, United States, [5]University of California Davis, CA, United States

All four serotypes of dengue virus (DENV) exist as quasispecies populations. Quasispecies are described as a spectrum of variants, genetically linked through mutation creating an interactive population where selection acts on the population rather than individual variants. While cloning provides linkage of all population mutations to single genomes; laborious methods are required to adequately sample the quasispecies population. High-throughput sequencing has become the method of choice in reconstructing quasispecies populations; though mutations within the population cannot be linked to single viral genomes. We examined the complexity and mutations discovered in clone-based quasispecies population analysis and compared results with assemblies obtained from the 454 sequencing. The E gene for 4 quasispecies populations from DENV-3 from a 2010 study in Thailand were cloned and sequenced using Sanger sequencing. Full genomes were obtained using 454 sequencing and E gene mutation comparisons with clones were conducted. The cloned populations, explored sequence space in several directions with transmission of dominant and subdominant variants (3-6 subdominant variants) and varying degrees of complexity. The percent of variants within the populations containing variable nt sites ranged from 68.9-77.6% (a.a. 46.7-57.1%) suggesting high population plasticity. Hinge region and non-functional variants were found in cloned populations however not in 454 assemblies due to low coverage. As read depth increased the probability of detecting cloned mutations increased. Full genome assemblies showed other potentially transmissible quasispecies mutations. Investigating dengue quasispecies diversity and behavior has relevance for understanding population responses to selective pressures such as innate and vaccine induced immunity. Work to investigate quasispecies population dynamics and complexity during illness and *in vivo*/vitro cycling using the MiSeq and PacBio systems to achieve high coverage and linkage of mutations to genomes within the population are underway.

1392

REAL-TIME FORECASTING OF THE 2014 DENGUE FEVER SEASON IN THAILAND

Nicholas G. Reich[1], Krzysztof Sakrejda[1], Derek A. Cummings[2], Paphanij Suangtho[3], Soawapak Hinjoy[3], Sopon Lamsirithaworn[3], Hannah Clapham[2], Henrik Salje[2], Justin Lessler[2]

[1]University of Massachusetts, Amherst, MA, United States, [2]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [3]Thai Ministry of Public Health, Bangkok, Thailand

Dengue is a major cause of morbidity in Thailand. Annual outbreaks of varying sizes provide a particular challenge to the public health system because treatment of severe cases requires significant resources. Advanced warning of increases in incidence could help public health authorities allocate resources more effectively and mitigate the impact of epidemics. In collaboration with the Thai Ministry of Public Health and Bureau of Epidemiology, we have developed a statistical model for infectious disease surveillance that uses data from across Thailand to give early warning of developing dengue epidemics. The model creates forecasts for each of the 77 Thai provinces. For each province, the forecast is based on (1) seasonal dynamics of dengue in the focal province, and (2) observed case counts at recent time-points from the focal province and neighbors demonstrated to be relevant through model selection using historical data. Prior to the beginning of the 2014 dengue season in Thailand, our team defined a process to generate forecasts for dengue in real-time. Beginning in April 2014, we created updated forecasts every two weeks based on the most current data from the Thai Ministry of Public Health database. We will present the results of this real-time forecasting exercise, including evaluating the performance of different forecasting models in predicting different features of the 2014 dengue season in each Thai province. Specifically, we will evaluate the ability of our models to predict the beginning, end, duration, and peak of the dengue epidemic. To our knowledge this is the first time that real-time forecasts of dengue have been attempted in Thailand based on reported case data.

1393

EXPLORING THE IMPACT OF INDIVIDUAL MOSQUITO SALIVARY PROTEINS ON DENV INFECTION IN THE VERTEBRATE

Michael K. McCracken, Rebecca C. Christofferson, Daniel M. Chisenhall, Britton J. Grasperge, Christopher N. Mores

Louisiana State University, Baton Rouge, LA, United States

Dengue virus (DENV) is transmitted during probing by an infectious mosquito concurrent with expectorated saliva. This saliva is composed of numerous proteins with anti-hemostatic and immuno-modulatory capabilities, and was shown previously to aid viral establishment within the vertebrate. IRF3/7 -/--/- (C57BL/6) mice intradermally-inoculated with DENV at sites of contemporaneous mosquito probing exhibited viremias of significantly enhanced magnitude and duration compared to mice unexposed to mosquitoes. This mosquito-driven enhancement was associated with differential regulation of immune transcripts involved in viral recognition and defense at early times post exposure. However, limited work exists on the relationship between individual *Aedes aegypti* salivary proteins and vertebrate infection with DENV. In an effort to characterize the contribution of individual salivary proteins to the enhancement of DENV infection, we have utilized recombinant salivary proteins for examination *in vivo*. One such protein was aegyptin, a known allergen and inhibitor of platelet aggregation. We intradermally-inoculated mice with and without co-inoculation of aegyptin and examined differences in viral titers and circulating leukocytes throughout viremia, along with viral titers and immune parameters at injection sites and draining lymph nodes at 48 hpi. Interestingly, co-inoculation of aegyptin resulted in decreased viral titers at inoculation sites and in circulation at 48 hpi compared to DENV alone, and these decreases were associated with alterations in cytokine concentrations in the lymph nodes of aegyptin-exposed mice. Additionally, co-inoculation of mice that had previously received multiple exposures to aegyptin resulted in further alterations to viremia titers. While co-inoculation of aegyptin did not yield universal enhancement of DENV titers, these results inform on immune pressures faced during DENV infection and support a complex system of interaction between the milieu of salivary proteins expectorated, DENV, and the vertebrate host environment.

1393

EXPLORING THE IMPACT OF INDIVIDUAL MOSQUITO SALIVARY PROTEINS ON DENV INFECTION IN THE VERTEBRATE

Michael K. McCracken, Rebecca C. Christofferson, Daniel M. Chisenhall, Britton J. Grasperge, Christopher N. Mores

Louisiana State University, Baton Rouge, LA, United States

Dengue virus (DENV) is transmitted during probing by an infectious mosquito concurrent with expectorated saliva. This saliva is composed of numerous proteins with anti-hemostatic and immuno-modulatory

capabilities, and was shown previously to aid viral establishment within the vertebrate. IRF3/7 -/--/- (C57BL/6) mice intradermally-inoculated with DENV at sites of contemporaneous mosquito probing exhibited viremias of significantly enhanced magnitude and duration compared to mice unexposed to mosquitoes. This mosquito-driven enhancement was associated with differential regulation of immune transcripts involved in viral recognition and defense at early times post exposure. However, limited work exists on the relationship between individual *Aedes aegypti* salivary proteins and vertebrate infection with DENV. In an effort to characterize the contribution of individual salivary proteins to the enhancement of DENV infection, we have utilized recombinant salivary proteins for examination *in vivo*. One such protein was aegyptin, a known allergen and inhibitor of platelet aggregation. We intradermally-inoculated mice with and without co-inoculation of aegyptin and examined differences in viral titers and circulating leukocytes throughout viremia, along with viral titers and immune parameters at injection sites and draining lymph nodes at 48 hpi. Interestingly, co-inoculation of aegyptin resulted in decreased viral titers at inoculation sites and in circulation at 48 hpi compared to DENV alone, and these decreases were associated with alterations in cytokine concentrations in the lymph nodes of aegyptin-exposed mice. Additionally, co-inoculation of mice that had previously received multiple exposures to aegyptin resulted in further alterations to viremia titers. While co-inoculation of aegyptin did not yield universal enhancement of DENV titers, these results inform on immune pressures faced during DENV infection and support a complex system of interaction between the milieu of salivary proteins expectorated, DENV, and the vertebrate host environment.

1394

EXTENDING A DETAILED *AEDES AEGYPTI* MODEL TO SIMULATE SINGLE AND COMBINED DENGUE CONTROL IN IQUITOS, PERU

Emily Griffiths, Mathieu Legros, Kenichi Okamoto, Fred Gould, Alun Lloyd

North Carolina State University, Raleigh, NC, United States

Attempts to control dengue include cleaning out water containers, poisoning larvae, and spraying insecticide inside homes. Other control measures like vaccines and engineered mosquitoes are being developed. We do not know which of these new control measures might result in the fewest dengue cases, either alone or in combination. We develop a complex simulation model that can compare single and multiple control interventions. This extends a detailed, stochastic model of *Aedes aegypti* population dynamics (Skeeter Buster) to include the movement patterns and infection histories of individual humans. We use this model to study the effect of various control measures on dengue epidemics in the city of Iquitos, Peru. We show how the speed and size of an epidemic varies with the number of places people visit each day. We also show the effects of spraying insecticide in homes, releasing dengue-resistant mosquitoes, and administering vaccinations. We test for single and pairwise combinations of these interventions, but find little evidence of synergistic effects. Our results suggest that combining control measures while making similar total investments may not prevent as many dengue cases as a single control measure.

1395

ADJUSTING UNDERREPORTED REAL TIME CASE DATA FOR PREDICTION OF DENGUE IN THAILAND

Krzysztof Sakrejda[1], Nicholas G. Reich[1], Derek A. Cummings[2], Paphanij Suangtho[3], Soawapak Hinjoy[3], Sopon Lamsirithaworn[3], Hannah Clapham[2], Henrik Salje[2]

[1]University of Massachusetts, Amherst, MA, United States, [2]Johns Hopkins University, Baltimore, MD, United States, [3]Ministry of Public Health, Nonthaburi, Thailand

Symptomatic cases of dengue virus, including dengue fever and dengue hemorrhagic fever, are an important cause of morbidity in Thailand. Thailand has a comprehensive nationwide case reporting system for Dengue and efforts are currently underway to leverage this data for real-time prediction of epidemic severity. Using case data for real-time prediction across a large geographic area that has multiple distinct administrative units is fraught with challenges. A central challenge is that the administrative units that contribute to the larger reporting system as a whole have heterogeneity in reporting processes which results in a substantial and highly variable interval of time (reporting interval) between when a case record is created and when it becomes available to use for prediction. Currently, dengue prediction models must use weeks-old case counts to assure that most relevant data has been processed through the reporting system. A better understanding of the reporting process in different locations could 1) provide metrics for use in optimizing the reporting system, and 2) make it possible to use the most recent incomplete counts for prediction of dengue epidemic intensity. We have developed and applied time-to-event models to characterize the spatial and temporal variation in reporting intervals and their relationship to case load and other seasonal features. Preliminary results suggest that this problem requires the use of contaminated time-to-event distributions to characterize reporting intervals and a hierarchical approach to combining information from diverse administrative units. We will present our analysis on the reporting process over the course of the 2013 and 2014 dengue seasons in Thailand, as well as our methods for improving the usability of real-time case data.

1396

DENV RNA AND ANTI-DENGUE ANTIBODY INTEGRITY IN CLINICAL SAMPLES ON DRIED BLOOD STABILIZATION PRODUCTS DURING AMBIENT TEMPERATURE SHIPMENT

Theron Gilliland, Jr.[1], Allison Dauner[1], Subhamoy Pal[1], Robert D. Hontz[2], Shuenn-Jue Wu[3]

[1]Naval Medical Research Center/Henry M. Jackson Foundation, Silver Spring, MD, United States, [2]U.S. Naval Medical Research Unit - 6, Lima, Peru, [3]Naval Medical Research Center, Silver Spring, MD, United States

Degradation of RNA and antibodies during specimen transport from collection site to diagnostic facility is a major problem affecting accurate diagnosis of RNA-based pathogens. This is particularly true when shipping may require more than a day of transit, as cold-chain is not always available in low-resource settings. In this study, we used dengue as a model RNA virus to compare the performance of three down-selected commercially available nucleic acid-stabilization products: Biomātrica DNAstāble tubes, ViveBio ViveST tubes, and Whatman FTA Micro Cards. Whole blood specimens collected from acute dengue fever patients (Days 0-4 Post Onset of Symptoms) during routine febrile surveillance in Iquitos, Peru were applied to the nucleic acid-stabilization products and dried overnight. At various time points, the stabilized specimens were shipped under ambient conditions (temperatures ranging from 9.7 to 34.3 °C and relative humidity ranging from 53.4 to 74.6% during shipment) to a diagnostic testing laboratory in Lima, Peru. Anti-dengue antibodies and dengue RNA levels were then tested via IgM ELISA and qRT-PCR, respectively, and compared to matched frozen unloaded controls. Agreements compared to each specimen's matched controls were: 97.3% IgM and 97.4% RNA (DNAstāble); 97.4% IgM and 95.0% RNA (ViveST);

and 81.6% IgM and 82.5% RNA (FTA Micro Cards).Other considerations such as cost, sample volume required, and ease-of-use were also evaluated in this study and should ultimately inform any decision to incorporate commercial sample stabilization products into a downstream diagnostic testing workflow.

1397

CHARACTERIZATION OF A DENGUE VIRUS TYPE 4 OUTBREAK IN SOUTH-CENTRAL MATO GROSSO, BRAZIL

Nayara Zuchi[1], Steven G. Widen[2], Rubing Chen[2], Hilda Guzman[2], Leticia B. Heinen[1], Sandra V. Mayer[2], Thomas G. Wood[2], Marcelo A. dos Santos[3], Jonathan E. Allen[4], Robert B. Tesh[2], Renata Dezengrini-Slhessarenko[1], **Nikos Vasilakis**[2]

[1]Federal University of Mato Grosso, Cuiabá, Brazil, [2]University of Texas Medical Branch Health, Galveston, TX, United States, [3]MT-Laboratory, State Secretary of Health, Cuiabá, Brazil, [4]Lawrence Livermore National Laboratory, Livermore, CA, United States

Dengue viruses (DENV) are by far the most important arboviral pathogens in the tropics globally, putting at risk of infection nearly a third of the global human population. In the current study we characterized the phylogeny and intrahost variation of 26 isolates of dengue virus type 4 (DENV-4) from acute serum samples obtained during an outbreak in South-Central Mato Grosso State (MT), Brazil, in 2012. All 26 isolates located within genotype II in two distinct lineages forming a monophyletic clade. Further confirmation of the co-circulation of two distinct lineages is obtained by analysis of the intrahost virus variation in the acute serum samples. Based on our phylogenetic analyses, there are 6 independent introductions of DENV-4 in Brazil, presumably from Venezuela, Puerto Rico, China, and Southeast Asia. The DENV-4 isolates of the 2012 outbreak in South-Central Mato Grosso State were closely related with two 2010 isolates from the geographically close regions of Amazonas and Roraima and were closely related with strains sampled from Venezuela 2007, indicating the potential origin introduction. The extent and severity of the 2012 DENV-4 outbreak is likely attributed to the lack of immunity in the population.

1398

CALL TO ACTION: A SCREENING TOOL FOR PREVENTION AND TREATMENT OF DENGUE IN TRAVELERS WITH CHRONIC COMORBIDITIES

Koya C. Allen

Kent State University, Kent, OH, United States

There is increased risk of Dengue Hemorrhagic Fever (DHF) in patients with comorbidities such as hypertension, diabetes, allergies and obesity. Lack of preparedness in epidemics can increase mortality rates amongst these patients when preexisting conditions are not identified and guidelines for case management not followed. In travelers, there is limited evidence for predicting clinical course of dengue in patients with chronic comorbidities. The WHO guidelines for treatment, prevention and control of dengue recommends identification of preexisting conditions and offers guidance for treating patients with obesity. Similar guidance is lacking for hypertension, diabetes and allergies. Acetylsalicylic acid (aspirin), Ibuprofen and other non-steroidal anti-inflammatory drugs (NSAID) are contraindicated for dengue; however, the prevalence of individuals on treatment regimens using these drugs is increasing. In an analysis of Behavioral Risk Factor Surveillance System (BRFSS) 2011 data, 25% of adults take aspirin daily and 31% have hypertension, of which 77% are being treated. In addition, 10%, 13% and 60% of adults had diabetes, asthma or obesity respectively. For travelers to dengue endemic regions it is important to develop and provide guidance for chronic disease management and dengue prevention. Post-travel it is important for medical professionals to have strict guidance on case management for potential dengue complications. A travel screening tool can identify high-risk travelers based on VFR status, Cultural Embeddedness, and social determinants of health in conjunction with comorbidities and current treatment. This tool can be implemented in pre-and post-travel consultation. Overall, this research recommends a call to action for researchers to 1) Develop guidelines for treating dengue patients with comorbidities; 2) Research effect of aspirin and NSAID regimens on dengue pathogenesis; and 3) Increase dengue surveillance and control in regions with high chronic disease prevalence and high population susceptibility to dengue, particularly travelers.

1399

INFLUENCE OF MATERNAL TOTAL IGG LEVELS ON TRANSPLACENTAL TRANSFER OF DENGUE VIRUS-SPECIFIC ANTIBODIES

Priscila M. Castanha[1], Eduardo J. Nascimento[1], Marli T. Cordeiro[2], Claudeir D. Silva Jr.[2], Ernesto T. Marques Jr.[1], Cynthia Braga[2]

[1]University of Pittsburgh, Pittsburgh, PA, United States, [2]Aggeu Magalhães Research Center, Recife, Brazil

Placental transfer of maternal dengue IgG antibodies to the fetus is likely to play an essential role in immunity and pathogenesis of dengue infection in infants. In order to investigate the kinetics of dengue-specific maternal antibodies transferred to children in the first two years of life, a birth cohort of 417 children living in an area of intense circulation of dengue virus in the northeast region of Brazil has been established. Here, we carried out a preliminary analysis of 216 dengue seropositive mother-newborn pairs to investigate the transference of total and dengue-specific IgG antibodies via placenta. Maternal and umbilical cord blood samples were obtained during the time of delivery. Serotype-specific antibody profile was determined by PRNT, while in-house ELISA was used to both measure dengue-specific IgG titers and estimate the levels of total IgG in the sera. Antibody titers were log-transformed and used to evaluate the degree of dengue-specific IgG transferred from mothers to infants. The average maternal age was 23.8 years (range, 13-41 years). In maternal sera, 127 out of 216 (58.8%) showed a monotypic profile against DENV3, 25.5% to the combination of DENV3/ DENV4 and 9.8% had detectable neutralizing antibodies against three or more serotypes. Dengue-specific IgG titers were significantly higher in cord blood (4.89±0.52) than in maternal samples (4.69±0.52; p=0.0006). A consistent pattern was also observed when comparing DENV3-specific PRNT titers in infants (2.68±0.83) and mothers (2.49±0.66; p=0.0095). Maternal levels of total IgG were negatively correlated with placental transfer of dengue-specific IgG (r= -0.1818, p= 0.0074) and DENV3 neutralizing antibodies (r= -0.1289, p=0.0586), indicating that very high levels of maternal IgG increases competition among the types of IgG transferred through the placenta. These results further suggest that maternal antibody transfer is influenced by maternal total IgG levels.

1400

CHARACTERIZING GLOBAL AND LOCAL TRENDS IN DENGUE TRANSMISSION: INSIGHT FROM AGE-SPECIFIC SURVEILLANCE DATA

Isabel Rodríguez Barraquer, Derek A. Cummings

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

Good characterization of global and local trends in dengue transmission has been challenging. Given that seroprevalence data, the gold standard to measure prior exposure, is very scarce, previous efforts have relied almost exclusively on total case or presence/absence data. However, while this approach has proven useful to define the distribution of dengue at a global scale, relying exclusively on counts may be misleading when looking at trends over time, or at finer spatial scales, due to the poor correlation that exists between infection and symptomatic disease. Here, we propose a framework to estimate yearly forces of infection (yearly probability of a susceptible individual being infected) and basic reproductive number

(R0) of dengue based on the age distribution of cases that are reported to surveillance systems. We use data from 4 countries where age-specific incidence data is publicly available (Thailand, Brazil, Mexico, Colombia) to estimate the force of infection and R0 over a period of 15 years at the province or, where possible, district levels. When available, we compare our estimates to those obtained from age-stratified serological surveys. Preliminary results suggest that age-specific incidence data provides a robust way to characterize dengue transmission at a global and local scale in settings of varying transmission intensity. In addition, they highlight the large heterogeneities in recent dengue epidemiology that exist within countries, provinces and probably even finer spatial scales. This is particularly true for countries such as Brazil, where dengue has recently re-emerged. Proper characterization of global and local trends in dengue epidemiology will be fundamental to target control interventions and design optimal vaccination strategies.

1401

MEDICAL COSTS OF DENGUE FEVER IN MEXICO

Luis Durán-Arenas, Adriana Zubieta-Zavala, Malaquías López-Cervantes

National Autonomous University of Mexico, Delegación Coyoacán, Mexico

In Mexico, dengue fever incidence has varied since its reappearance in 1970s, with peaks in 1980, 1997, and 2009 and >130 000 cases. With high incidences, accurate cost estimates of disease are needed to efficiently use finite treatment and prevention health resources (vaccination and vector control). This study assessed medical cost and cost to the infected individual using a micro-costing approach to overcome a lack of centralized data. The cost per dengue case is derived from health system direct medical costs, patient direct costs, and productivity loss-related indirect costs. Costs were calculated in the SS and the IMSS settings. To derive health system costs, an ideal protocol for dengue fever treatment was based on a review of national and international norms, guidelines, and expert consensus combined with a microcosting tool known as PAATI (program, actions, activities, tasks, inputs). For comparison to real costs, actual tasks and inputs for real dengue fever cases were derived from chart review and health personnel and hospital administrators interviews. Patient direct and indirect costs were derived from patient interviews. Indirect cost was defined as disease-associated productivity loss (to patient and carer). Of chart reviews (N=1440) foreseen in 18 Mexican states, 1293 were obtained (90%) and clinical pathways were obtained for 1168 (81%). For direct medical costs, we observed an increased cost gradient depending setting (ambulatory $92 USD, hospitalized $1644, ICU $9375). We noted a difference between ideal cost and real cost in both SS and IMSS systems. The main difference driver in ideal costs between outpatients and hospitalized patients was cost of professional services (~90% for outpatients and ~100% for hospitalized/ICU patients). Medicine accounts for a fraction of overall cost, yet real expenditure is reduced compared to ideal expenditure for drugs. Direct real medical cost of ambulatory cases of $33 for SS is lower than direct medical costs reported in Brazil ($49),Colombia ($67) whereas medical cost for IMSS system ($92) is higher and comparable to Venezuela ($118). In contrast, hospitalized patient direct medical cost, in relative terms, is higher: $490 and $1644 in SS and IMSS respectively, vs $318 for Brazil, $331 for Columbia ($864 for Venezuela). Real costs and costs associated with ideal treatment are different, particularly for outpatients, pointing to health system failings (both SS and IMSS).

1402

EARLY INDICATORS OF DENGUE AMONG CHILDREN AND ADULTS PRESENTING WITH ACUTE FEBRILE ILLNESS IN PUERTO RICO

Doris A. Andujar-Perez[1], Olga D. Lorenzi-Pena[2], Kay M. Tomashek[2], Frances Gonzalez-Figueroa[3], R. Ivan Iriarte[3], Luisa I. Alvarado-Domenech[3]

[1]Ponce School of Medicine and Health Sciences/Saint Luke's Episcopal Hospital, Ponce, PR, United States, [2]Centers for Disease Control and Prevention, Division of Vector-Borne Disease - Dengue Branch, San Juan, PR, United States, [3]Ponce School of Medicine and Health Sciences/Saint Luke's Episcopal Hospital, Ponce, PR, United States

Early clinical diagnosis of dengue can be challenging because the initial presentation is nonspecific with signs and symptoms similar to those of other acute febrile illnesses (AFI). Rapid diagnostic testing is often not available. Early identification and timely initiation of correct treatment can reduce complications and mortality. To identify early indicators for laboratory-positive dengue, we analyzed data from a sentinel enhanced dengue surveillance system conducted at a large referral hospital in Puerto Rico. Outpatients with fever for <7 days were enrolled and followed through their illness. Serum and nasopharyngeal specimens were collected and tested by RT-PCR and immunodiagnostic methods as appropriate for dengue viruses (DENV-1-4), *Leptospira* spp., *Burkholderia pseudomallei*, 5 enteroviruses, influenza A and B viruses, and 12 other respiratory viruses. Laboratory-indeterminate cases, co-infections and infants were excluded from analysis. Among the 1,580 patients enrolled during May 7, 2012 through May 6, 2013, 570 (36.1%) were hospitalized, 805 (51.0%) were male, and the median age was 21.1 years (range: 1-91 years). There were 617 dengue-positive patients, 611 respiratory infections, 72 infections caused by other viruses or bacteria and 280 cases with no pathogen identified. Five clinical findings were found to be independently associated with a laboratory-positive dengue: retrorbital pain, leukopenia, thrombocytopenia, rash and facial erythema. Sore throat, nasal congestion and cough were less frequent on dengue-positive patients. Clinical and laboratory features that were predictive of dengue were found to vary by patient age. Dengue was associated with: leukopenia, rash and joint pain ($p<0.005$) in children aged <9 years; leukopenia and thrombocytopenia ($p < 0.005$) in individuals aged 10-19 years; and thrombocytopenia, leukopenia, rash, nausea and joint pain ($p < 0.003$) in adults aged ≥20 years. Knowledge of predictors can be used to direct anticipatory guidance.

1403

PRESENCE OF THREE DENGUE SEROTYPES IN OUAGADOUGOU, BURKINA FASO AND ITS PUBLIC HEALTH IMPLICATIONS

Beatriz Parra[1], **Mabel Carabali**[2], Antarou Ly[3], Emmanuel Bonnet[4], Florence Fournet[5], Roch K. Dabiré[6], Clara Beatriz Ocampo[7], Seni Kuanda[8], Isabelle Agier[9], Valéry Ridde[10]

[1]Universidad del Valle, Cali, Colombia, [2]Dengue Vaccines Initiative/ International Vaccines Institute, Seoul, Republic of Korea, [3]Institut de Recherche en Sciences de la Santé, IRSS/CNRST, Ouagadougou, Burkina Faso, [4]Institut de Recherche pour le Développement, Identités et Différenciations de l'Environnement des Espaces et des Sociétés, Paris/ Caen, France, [5]IRSS/Centre Muraz, Bobo-Dioulasso, Burkina Faso/UMR MIVEGEC Institut de Recherche pour le Développement, Montpellier, France, [6]IRSS/Centre Muraz, Bobo-Dioulasso, Burkina Faso, [7]Centro Internacional de Entrenamiento e Investigaciones Medicas, Cali, Colombia, [8]Institut de Recherche en Sciences de la Santé, CNRST, Ouagadougou, Burkina Faso, [9]University of Montreal Hospital Research Centre (CRCHUM), Montreal, QC, Canada, [10]University of Montreal Hospital Research Centre (CRCHUM)/School of Public Health (ESPUM), University of Montreal, Montreal, QC, Canada

The discussion about the presence of febrile non-malaria cases has increased in Burkina Faso. As other febrile diseases, dengue was considered as differential diagnosis due the presence of the vector and previous DENV reports in the country. To explore the virus presence in acute febrile non-malaria cases and *Aedes* mosquitoes in Ouagadougou, an exploratory cross sectional study was performed from December 2013 to January 2014. Five sectors and six correspondent health care centers (CSPS) were selected based on a reported presence of Flavivirus: CSPS 3 and 12 (Dapoya), 8 (Gounghin), 18 (Pissy), 25 (Somgandé) and 28 (Dassasgho). A survey about symptoms was administered to the participants and finger pricks were used to obtain the samples. Each CSPS tested every febrile non-malaria patient for dengue using dengue rapid tests (SD Bioline DengueDuo). Blood spots were obtained in filter paper from all positive results and every tenth negative for further PCR analyses. A parallel entomological survey was conducted in the CSPS's correspondent sectors. From a total of 379 patients tested, 35 (9.2%) were positive for rapid test (60% both IgM/IgG; 21% just IgG and 5% just NS1). 91% were older than 15 years old (range 0-61 years old), 60% were women and 70% came to the CSPS during the first 3 days of fever. From 60 samples tested by RT-PCR, 15 were positive (9 from positive rapid test and 6 from the subsample of negative results). The serotypes observed were DENV2 (Dassasgho and Gounghin), DENV3 (Dapoya, Pissy and Somgande) and DENV4 (Dapoya, Gounghin and Somgande). There was not DENV in the analyzed mosquitoes. The presence of dengue in acute febrile non-malaria patients in Ouagadougou was evidenced. To our knowledge, thought the presence of DENV3 and DENV4 were reported in the region, this is the first time both serotypes are evidenced in Burkina Faso. These findings have important public health implications due the need to prepare the health system and the population for dengue's presence and outbreaks prevention (Additional data will be available at the conference)

1404

EVIDENCE OF RECENT DENGUE EXPOSURE AMONG MALARIA PARASITE-POSITIVE CHILDREN IN THREE URBAN CENTERS IN GHANA

Justin Stoler[1], Rupert K. Delimini[2], J. H. Kofi Bonney[3], Gordon A. Awandare[2]

[1]University of Miami, Coral Gables, FL, United States, [2]University of Ghana, Legon, Ghana, [3]Noguchi Memorial Institute for Medical Research, Legon, Ghana

Dengue fever is increasingly being recognized as an important neglected tropical disease in sub-Saharan Africa, with national burdens generally unknown due to misdiagnosis of cases as malaria. This study screened for evidence of dengue exposure in 222 children aged 2-14 living in Accra, Kintampo, and Navrongo, Ghana, who tested positive on a rapid diagnostic test (RDT) for malaria and were subsequently confirmed to be malaria parasite-positive via blood test. We found presence of dengue-specific IgM antibodies using indirect ELISA methods in 7 children screened across the three sites, and presence of dengue-specific IgG antibodies in 20%, 13%, and 30% for Accra, Kintampo, and Navrongo respectively. The high rates of dengue exposure among children with confirmed malaria may be just the tip of the iceberg in terms of dengue prevalence among the heavy volume of febrile illness patients who do not have confirmed malaria. We discuss demographic correlates of dengue exposure and argue that this study underscores the need for assessing Ghana's baseline dengue burden as well as general clinical knowledge of the disease.

1405

CO-INFECTION WITH DENGUE AND RESPIRATORY VIRUSES AMONG CHILDREN WITH ACUTE FEBRILE ILLNESS, PUERTO RICO

Yajaira Guzman[1], Olga D. Lorenzi[2], Xyomara Jimenez[1], Maria Aranda[1], N. Judith Lopez[1], Ivonne Galarza[1], Jorge L. Muñoz-Jordán[2], Elizabeth Hunsperger[2], Harold S. Margolis[2], Ivan Iriarte[3], Luisa I. Alvarado[1]

[1]Hospital Episcopal San Lucas, Ponce, Puerto Rico, [2]Dengue Branch, Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, San Juan, Puerto Rico, [3]Public Health Program, Ponce School of Medicine and Health Sciences, Ponce, Puerto Rico

Dengue is endemic in Puerto Rico with seasonal increases in incidence that often coincide with increases in other acute febrile illnesses (AFI) caused by respiratory pathogens. Consequently, co-infections are possible, which may complicate both diagnosis upon presentation and the patients' clinical course. Specifically, the presence of respiratory symptoms may reduce early diagnosis of dengue, which is important for early initiation of clinical management that can minimize medical complications and mortality. In May 2012, a sentinel enhanced dengue surveillance system (SEDSS) site was established in a tertiary care hospital in Ponce, Puerto Rico wherein patients with fever for <7 days were enrolled and followed through their illness. Serum, nasopharyngeal and oropharyngeal specimens were collected and tested by RT-PCR for multiple pathogens including: dengue virus subtypes 1-4 (DENV-1-4); influenza A and B viruses, adenovirus, respiratory syncytial virus, metapneumovirus, and parainfluenza viruses. To identify factors associated with co-infection patients with DENV and respiratory virus co-infection (cases) were age-matched to patients infected with DENV only (controls) at a ratio of 1:2. Of 715 case-patients with DENV detected in serum, 30 (4.2%) had evidence of co-infection with a respiratory virus. There were no differences by gender identified among cases and controls. Most (87%) of the co-infections were children and adolescents (<20 years). Cases were more likely than controls to report cough (odds ratio [OR] = 3.17; 95% confidence interval [CI]: 1.2-8.1) or runny nose (OR = 2.58; 95% CI: 1.02, 6.50). Cases were also more likely to have a chest x-rays ordered, although this difference was not statistically significant (OR =1.6; 95% CI: 0.61-4.0). Further analysis will include factors associated with illness severity and clinical outcome. These findings suggest that in areas with endemic dengue and respiratory pathogens, physicians should have a high index of suspicion for co-infections in children and adolescents.

1406

POSITIVE SEROLOGY FOR HANTAVIRUS IN PATIENTS WITH CLINICAL SUSPECTED DENGUE IN CEARÁ, BRAZIL

Danielle M. Lima[1], Raissa M. Fontes[2], Gilberto S. Santos Junior[3], Jeová K. Colares[1], Benedito A. Fonseca[3], Luiz T. Figueiredo[3]

[1]University of Fortaleza, Fortaleza, Brazil, [2]Federal University of Ceará, Fortaleza, Brazil, [3]School of Medicine of Ribeirão Preto-USP, Ribeirão Preto, Brazil

Dengue is considered the most important arbovirus in the world in terms of morbidity and mortality, having broad and unspecific symptoms, ranging from asymptomatic to severe hemorrhagic forms. Thus, it becomes difficult to distinguish it from other febrile syndromes only by clinical and epidemiological criteria. The use of these criteria as the unique basis for diagnosis of dengue can be dangerous and may lead to false diagnoses and inappropriate treatment. Within of the spectrum of similar to dengue acute febrile diseases, some pathogens are not routinely investigated by the lack of resources and ignorance of their existence in the region. In Ceará, the hantavirus was never notified and there is only one report in the regional literature showing the probable existence of this disease in humans in the state. Thus, the aim of this study was to investigate cases of hantavirus in patients suspected of dengue in Ceará. In this study, we evaluated 95 patients, with clinical suspicion of dengue, recruited during the year 2012 in the State of Ceará. The samples were evaluated for hantavirus through ELISA-IgM and ELISA-IgG tests. One (1.05%) patient was positive for hantavirus by ELISA- IgM, detecting current or recent infection by the virus. This patient had moderate symptoms, suggesting that mild or atypical cases of hantavirus should be occurring in the State. Thirty (31.6%) patients were positive by ELISA-IgG. This result suggests that they have recently or previously infected by hantavirus, but this result does not allow to determine whether this virus was the causative agent of febrile syndrome presented by these patients. All patients in this study were questioned about the conduct of recent trips and none reported having left Ceará in recent months, probably acquired the infection locally. For a state that has only one report in the literature for this pathogen in humans, the percentage of people with prior contact with it was very high, showing the need for further investment and research on this disease in Ceará. Financial support: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

1407

DESCRIPTION OF FEBRILE ILLNESS AND DENGUE IN INFANTS LESS THAN 90 DAYS OLD

Mariana Tavárez[1], Janice Perez-Padilla[2], N. Judith Lopez[1], Ivan Iriarte[3], Luisa I. Alvarado[1]

[1]Hospital Episcopal San Lucas, Ponce, Puerto Rico, [2]Dengue Branch, Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, San Juan, Puerto Rico, [3]Public Health Program, Ponce School of Medicine and Health Sciences, Ponce, Puerto Rico

Fever in the first 90 days of life presents a diagnostic and therapeutic challenge for pediatricians. Bacterial infections should be identified in order to provide adequate treatment, but sepsis workup is invasive and costly. Differentiation between bacterial and viral etiology is important to prevent unnecessary invasive procedures. Viruses, including dengue (DENV), are believed to be an important cause of fever in endemic countries, but they are not routinely identified and knowledge of their contribution to febrile illness in young infants is limited. This study used data obtained from the Sentinel Enhanced Dengue Surveillance System (SEDSS) established in southern Puerto Rico in May 2012. SEDSS recruits acute febrile illness (AFI) patients, collects clinical data and tests for 22 infectious agents, including DENV, respiratory pathogens and enteroviruses. Reverse transcriptase--polymerase chain reaction and ELISA are used to identify etiologic agents. Of 5,115 patients enrolled during the first year of SEDSS, 48 (0.9%) were infants less than 90 days old. Thirty (62.5%) infants were male, and 9 (18.8%) were less than 30 days old. Twenty four (50%) infants presented on the first day of fever, and most (89.4%) presented within the first 3 days. Most infants (70.8%) were admitted for cultures and treatment, including all patients less than 30 days old. The etiologic agent was identified in 13 (27%) infants: 3 (6.3%) had a bacterial infection, 8 (16.7%) had a viral infection, and 2 (4.2%) had viral/bacterial co-infection. Viruses detected included DENV (n = 2), influenza A virus (n = 4), enterovirus (n = 2), parainfluenza virus-3 (n = 1), and DENV/influenza A virus coinfection (n = 1). Viral infections were more common than bacterial infections in this pediatric cohort, and DENV infection was a rare event. These findings will assist clinicians to understand the causes of fever and the incidence of DENV infection in young infants.

1408

DENGUE VIRUS TYPE 3 CIRCULATION IN A REGION OF THE COLOMBIAN CARIBBEAN DURING AN EPIDEMIC PERIOD

Erwin Camacho-Burgos, Homer Corrales, Sindy Martínez, Kelly Villadiego, Pedro Blanco-Tuirán

Universidad de Sucre, Sincelejo, Colombia

Dengue is an arthropod-borne viral disease which has become a major international public health problem in terms of economic impact, mobidity and mortality. Dengue virus 3 is a recently introduced serotype in Colombia which in addition to the simultaneous circulation of the other serotypes, could be associated with an increased transmission and appearance of severe manifestations risk; however, the local virus surveillance in areas such as the department of Sucre is not constant, leading to the lack of updated information. In the present study we describe the frequency of circulation and phylogenetic characteristics of Dengue virus type 3, present in the department of Sucre, located in the Colombian Caribbean. Clinical data and blood samples from patients with febrile syndrome were collected during the second half of 2013 and early 2014. Molecular detection of DENV was performed by a One-Step RT-PCR and IgM/IgG antibodies against the virus were determined by a capture ELISA. Two C6/36 cell passages were made with the RT-PCR positive samples, for virus isolation. Supernatants were used to amplify the complete E and NS3 genes to be subsequently sequenced in order to perform phylogenetic analysis (Bayesian Inference). 22% of the samples were positive for molecular detection of the virus, whereas 37.7 %, 11.1%, and 24% had IgM, IgG and IgM/IgG antibodies against DENV respectively. Serotypes DENV1, 2 and 3 were detected but DENV3 was the most frequent (60%). Three isolates were obtained corresponding to DENV3 (2) and DENV1 (1). The sequence analysis revealed high similarities between DENV3 isolates that were classified within the genotype III; DENV1 isolate was classified as American/African genotype closely related with Colombian and Venezuelan sequences. The results suggest that most of the Dengue reported cases during this epidemic period were caused mainly by DEN3, but other two serotypes were present. This confirm that the region is a hyperendemic area, which could potentially be a hotspot for Dengue transmission in the Colombian Caribbean.

1409

SENSITIVITY AND SPECIFICITY OF THE WORLD HEALTH ORGANIZATION DENGUE CLASSIFICATION SCHEMES FOR SEVERE DENGUE ASSESSMENT IN CHILDREN IN RIO DE JANEIRO

Gleicy A. Macedo[1], Michelle L. Gonin[2], Sheila M. Pone[1], Oswaldo G. Cruz[1], Flávio F. Nobre[3], **Patrícia Brasil**[1]

[1]Fundação Oswaldo Cruz-Fiocruz, Rio de Janeiro, Brazil, [2]Hospital Municipal Jesus, Rio de Janeiro, Brazil, [3]Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

The clinical definition of severe dengue fever remains a challenge for researchers in hyperendemic areas like Brazil. The ability of the traditional (1997) as well as the revised (2009) World Health Organization (WHO) dengue case classification schemes to detect severe dengue cases was

evaluated in 267 children admitted to hospital with laboratory-confirmed dengue. Using the traditional scheme, 28.5% of patients could not be assigned to any category, while the revised scheme categorized all patients. Intensive therapeutic interventions were used as the reference standard to evaluate the ability of both the traditional and revised schemes to detect severe dengue cases. Analyses of the classified cases (n = 183) demonstrated that the revised scheme had better sensitivity (86.8%, P < 0.001), while the traditional scheme had better specificity (93.4%, P < 0.001) for the detection of severe forms of dengue. This improved sensitivity of the revised scheme allows for better case capture and increased ICU admission, which may aid pediatricians in avoiding deaths due to severe dengue among children, but in turn, it may also result in the misclassification of the patients' condition as severe, reflected in the observed lower positive predictive value (61.6%, P < 0.001) when compared with the traditional scheme (82.6%, P < 0.001). The inclusion of unusual dengue manifestations in the revised scheme has not shifted the emphasis from the most important aspects of dengue disease and the major factors contributing to fatality in this study: shock with consequent organ dysfunction.

1410

EVALUATION OF TWO PARAMETERS FOR DENGUE DIAGNOSIS IN HONDURAN PATIENTS

Leda A. Parham, Carlos Sánchez, María José López, Ivette Lorenzana

Microbiology Department, National Autonomous University of Honduras, Hospital Materno Infantil, Tegucigalpa, Honduras

Dengue is an important vector borne disease in tropical and sub-tropical countries. In Honduras during year 2013, around 39,275 cases of dengue fever were registered at national level. Several approaches have been developed for laboratory diagnosis of dengue infections, nevertheless timely diagnosis is a challenge. This study was undertaken to evaluate two different tests to detect dengue virus NS1 antigen (Ag) and dengue IgM antibodies (Ab) manufactured by Standard Diagnostics (SD, South Korea) in samples from patients with dengue infections. The study was carried out in Tegucigalpa, Honduras. The study population consisted of 134 patients clinically classified with dengue hemorrhagic fever according to the WHO criteria. Out of 134 plasma samples, 61 corresponded to patients with ≤5 days of illness and 73 samples to patients with ≥6 days of illness. All samples from patients with ≤5 days of illness, characterized as dengue positive (n=48) or negative (n=13) by RT-PCR, were tested by SD dengue NS1-Ag methods (the rapid test SD Bioline NS1-Ag and SD NS1-Ag EIA); all samples from patients with ≥6 days of illness with positive (n=57) or negative (n=16) result for dengue infection by an in-house IgM-Ab capture EIA, were tested by SD dengue IgM-Ab methods: the rapid test SD Bioline IgM-Ab and SD IgM-Ab EIA. The sensitivity of SD Bioline NS1-Ag was 88% and 85% for SD NS1-Ag EIA. Regarding specificity, although it is 17% for SD Bioline NS1-Ag and 23% for SD NS1-Ag EIA this is not real, the comparison was done with RT-PCR and turn out to be false negative; because 9/10 samples are IgM-Ab positive. For SD IgM-Ab, the sensitivity and specificity was 82% and 88% for SD Bioline IgM-Ab and 88% and 69% for SD IgM-Ab EIA when were compared with the in-house EIA. These results suggest that dengue SD Bioline NS1-Ag method had slightly higher sensitivity than dengue SD NS1-Ag EIA (88% vs 85%). Higher specificity was observed for SD Bioline IgM-Ab than SD IgM-Ab EIA (88% vs 69%). In terms of sensitivity SD IgM-Ab EIA was higher than SD Bioline IgM-Ab (88% vs 82%). Early diagnosis of dengue infection by NS1 antigen could be helpful in the timely management of dengue virus infection, and it might be even superior due to the fact of the known liability of RNA used for molecular testing and confirmed in this study.

1411

SPATIOTEMPORAL CLUSTERING, CLIMATE AND SOCIAL-ECOLOGICAL RISK FACTORS FOR DENGUE IN MACHALA, ECUADOR

Anna M. Stewart Ibarra[1], Angel Munoz[2], Keytia Rivero[3], Efrain Beltran[4], Mercy J. Borbor[5], Julia Finkelstein[6], Raul Mejia[3], Tania Ordonez[4], Cristina Recalde[3], Daniel Ruiz[7], Sadie J. Ryan[8]

[1]SUNY Upstate Medical University, Syracuse, NY, United States, [2]Columbia University, New York, NY, United States, [3]National Institute of Meterology and Hydrology, Guayaquil, Ecuador, [4]Ministry of Health, Machala, Ecuador, [5]Escuela Superior Politecnica del Littoral, Guayaquil, Ecuador, [6]Cornell University, Ithaca, NY, United States, [7]University of Antioquia, Medellin, Colombia, [8]University of Florida, Gainesville, FL, United States

Dengue fever, a mosquito-borne viral disease, is a growing public health problem in Ecuador and throughout the tropics, yet we have a limited understanding of the disease dynamics in these newly emerging regions. The aim of this study was to characterize the spatiotemporal dynamics, climate and social-ecological risk factors associated with the largest dengue outbreak on record (2010) in the coastal port city of Machala, Ecuador. Spatial analysis: Using LISA and Moran's I, we analyzed the spatial distribution of georeferenced dengue cases and found evidence of significant hotspots near the city center. We evaluated whether the presence of dengue transmission was associated with social-ecological variables at the neighborhood level by overlaying data from the 2010 national census and entomological indices. We used a multi-model selection process and found that the best-fit model to predict the presence of dengue included age and gender of the head of the household (older, female), access to piped water in the home, poor housing condition, and distance to the central hospital. Temporal analysis: Using wavelet analysis, we characterized historical patterns of weekly climate and dengue transmission (2003-2010), and we found significant climate effects associated with the outbreak. In conclusion, our findings indicate the potential to develop dengue vulnerability maps that can feed into climate-driven dengue early warning systems to inform vector control interventions. This study provides an operational methodological framework that can be broadly applied to understand local dengue risk.

1412

THE COMPLEX RELATIONSHIP BETWEEN WEATHER AND DENGUE VIRUS TRANSMISSION IN THAILAND AND PERU

Karen M. Campbell

San Diego State University, San Diego, CA, United States

Dengue viruses cause more human morbidity and mortality than any other arthropod-borne virus. Dynamic space-time estimations of risk are needed to guide the development of more effective surveillance-intervention strategies and use of prevention resources. Weather plays an important role in regulating the location and timing of transmission due to direct effects of temperature and humidity on mosquito development cycles, life span, behavior and extrinsic incubation period. We closely examined the relationship between weather dynamics, including temperature, humidity, and rainfall, and dengue virus transmission across all of Thailand by province for 1983-2001 and all of Peru by district for 1994-2012. We quantitatively characterized the role of weather in regulating dengue transmission cycles across both countries. We observed systematic differences in the structure of seasonal transmission cycles of different magnitude, the role of weather in regulating seasonal cycles, necessary versus optimal transmission "weather-space", basis of large epidemics, and predictive indicators that estimate risk. Larger epidemics begin earlier, develop faster and are predicted at seasonal *Onset change-point* when case-counts are low. Temperature defines a viable range for transmission; humidity amplifies the potential within that range. This duality is central to transmission and epidemic magnitude. In Thailand, 80% of 1.2 million severe dengue cases occurred when mean-temperature was 27--29.5°C and mean-humidity was >75%. In Peru, with highly diverse weather

patterns spatially, broadly relevant predictors were developed using a statistical classification approach. Interventions are most effective when potentially large epidemics are identified early. Most cases occur near the local seasonal *Peak*, yet small reductions at epidemic *Onset* can substantially reduce epidemic magnitude. Monitoring the *Quiet-Phase* before *Onset* is fundamental in effectively targeting interventions pre-emptively.

1413

ESTIMATING CROSS-IMMUNITY INTERACTIONS OF DENGUE SEROTYPES USING LONGITUDINAL SEROLOGICAL DATA

Robert C. Reiner[1], Steven Stoddard[1], Brett Forshey[1], Eric Halsey[2], Amy Morrison[1], Tadeusz Kochel[2], Thomas Scott[1]

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

Dengue, a mosquito-borne disease whose incidence and graphic range have increased considerably in the past 50 years, is caused by any of four related but antigenically distinct virus serotypes (DENV-1, DENV-2, DENV-3, DENV-4). Following a DENV infection, in addition to lifelong immunity to the infecting serotype, an individual gains temporary immunity to infections with heterologous viruses. Although temporary cross-immunity (TCI) is a historically demonstrated phenomenon, the strength and duration of this vital component of DENV epidemiology is difficult to estimate. Critically, TCI's primary role in transmission dynamics results in the absence of heterologous infections; something that cannot be explicitly captured using hospital case data. Conversely, large longitudinal serological surveys from the same subset of the population over the course of several years can be used to estimate the risk each individual faces for infection with each serotype and observe the disproportionate decrease (or complete absence) of heterologous infections immediately following a DENV infection. Here we apply a new modeling approach that estimates TCI using a 12-year longitudinal DENV dataset from Iquitos, Peru. The dataset contained information on 14,335 individuals whose blood was assayed by PRNT every 6-9 months (38,416 total samples), and contained interval censored timing for 3,854 serotype-specific infections. We identified 455 individuals that became infected with two different serotypes during their participation in the study. Although the average time between seroconversions was 449 days (2.5 tests on average), 250 of those individuals seroconverted twice in sequential assays. Further analysis, using a spline-based approach previously designed to study the serotypes independently, is currently being leveraged to resolve when, within these testing intervals, infections likely occurred. By modeling a variety of ranges and distributions for the length of cross-immunity, we can estimate the strength of the interactions between DENV serotypes with greater accuracy than was previously possible.

1414

MECHANISMS OF TRAVELING WAVES AND PERIODIC SPATIAL SYNCHRONIZATION OF DENGUE HEMORRHAGIC FEVER INCIDENCE IN THAILAND

Luis Mier-y-Teran[1], Kate Grabowski[1], Justin Lessler[1], Henrik Salje[1], Isabel Rodriguez-Barraquer[1], Donald Burke[2], Surapee Anantapreecha[3], Atchareeya A-Nuegoonpipat[3], Richard Jarman[4], Sopon Iamsirithaworn[5], Simone Bianco[6], Leah B. Shaw[7], Ira B. Schwartz[8], Derek A. Cummings[1]

[1]Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States, [2]University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA, United States, [3]National Institute of Health, Department of Medical Sciences, Bangkok, Thailand, [4]Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [5]Bureau of Epidemiology, Department of Diseases Control, Ministry of Public Health, Bangkok, Thailand, [6]University of California San Francisco, San Francisco, CA, United States, [7]College of William and Mary, Williamsburg, VA, United States, [8]Plasma Physics Division, Naval Research Laboratory, Washington, DC, United States

Multi-annual periodicity has been observed in multiple time series of dengue including those from Thailand. These time series have been observed to have distinct spatial structuring of the timing of peaks, with spatio-temporal traveling waves and other structures observed. The mechanisms underlying this spatial dependence are not well understood. Here we describe transmission models that explore multiple hypotheses of the mechanism underlying traveling waves and periodic synchronization of dengue incidence observed in Thailand in a 40 year time series of incidence from all provinces in the country. We utilize mechanistic, meta-population models that include migration between patches to understand the incidence synchronization phenomenon. We explore scenarios with varying degrees of patch heterogeneity, migration rates, seasonal forcing and heterogeneity in birth rates to identify the main drivers behind phase structures and synchronization observed in the empirical incidence data. We discuss the potential impact of our observations for the control of dengue.

1415

DENGUE VIRUS INFECTION AMONG MEMBERS OF THE UGANDA PEOPLE'S DEFENSE FORCE

Elizabeth Hunsperger[1], Esther M. Ellis[1], D. Fermin Arguello[1], Joel Montgomery[2], Jeff Borchert[3], John Kayiwa[4], Lutwana Julius Julian[4], Jessica Carrion[1], Kay M. Tomashek[1], Harold Margolis[1]

[1]Centers for Disease Control and Prevention, San Juan, PR, United States, [2]Centers for Disease Control and Prevention, Division of Global Health Protection, GA, United States, [3]Centers for Disease Control and Prevention, Uganda, Uganda, [4]Uganda Virus Research Institute, Uganda, Uganda

Outbreaks of dengue have occurred in East Africa over the last several years. In May 2011, a dengue outbreak was recognized among African Union Mission in Somalia (AMISOM) peacekeepers when several Ugandans were diagnosed with an acute hemorrhagic febrile illness. In response to the outbreak, we conducted a seroincidence study to determine the risk of dengue virus (DENV) infection following Uganda Peoples Defense Force (UPDF) deployment to Somalia. Serum specimens were obtained from 337 participating UPDF soldiers to determine DENV exposure and infection rate pre- and post- deployment. Testing included anti-DENV IgG antibodies by immunoassay and neutralizing IgG antibodies by microneutralization test (MNT). A dengue case was defined as positive for IgG seroconversion and confirmed by MNT. IgG seroconversion was defined as a negative anti-DENV IgG result in the pre-deployment specimen and a positive result in the post-deployment specimen or a 4-fold titer increase. MNT positive titer to only one serotype was classified as a primary DENV infection. Reactivity to multiple DENV serotypes by MNT was classified as a secondary DENV infection. Sixty percent of the UPDF soldiers that deployed to Somalia

had seroconversion by IgG. The MNT results showed that 81% of the IgG positive specimens had neutralizing antibodies specific to DENV. Only 13.3% of the IgG positive specimens had a primary infection to either DENV1, 2 or 3. DENV-3 was the predominant serotype amongst UPDF soldiers. DENV exposure determined by the seroincidence study following UPDF deployment to Somalia matched the identified circulating serotypes in Somalia during the dengue outbreak in 2011. With dengue in the differential diagnosis for acute febrile illness for the UPDF soldiers, a quarantine recommendation should be considered for returning soldiers so as not to introduce DENV into Uganda.

1416

MOLECULAR CHARACTERIZATION OF INFLUENZA A AND B VIRUSES IN CUBA DURING 2006-2010, IMMUNOLOGICAL MARKERS RELATED TO 2009 PANDEMIC DISEASE SEVERITY

Alexander Piñón, Belsy Acosta, Odalys Valdés, Amely Arencibia, Mayra Muné, Yamila Adams, Clara Savón, Suset Oropesa, Angel Goyenechea, Grehete González, Guelsys González

Tropical Medicine Institute Pedro Kourí, Havana, Cuba

During 2006-2010 in Cuba, Influenza and pneumonia were the fourth leading cause of death, with the detection of pandemic influenza A(H1N1) pdm09 in year 2009. Severe disease caused by pandemic virus in persons less than 55 years occurred at highest frequencies than youngest and elder groups worldwide. At that time, scientific community focused on the interaction virus-ecology-host factors. Besides, emergency of genetic variants divergent from vaccine strains and antiviral drug-resistant variants, threaten the effectiveness of prevention and control measures. In Cuba, there are non-previous studies about influenza viruses molecular characterization, we focused in genetic characterization of influenza A and B virus variants circulating during 2006-2010. In addition, the relationship between Influenza pandemic severity with host factors was determined. Study showed seasonal influenza A and B viruses into different genetic variants, some of them genetically divergent from vaccine strain. Different genetic variants of influenza virus A(H1N1) pdm09 were detected, however, they remain the genetic match with vaccine strain. High levels of RANTES and TLR-2, and the presence of CCR5Δ32 suggest their involvement in disease severity produced by the 2009 pandemic virus. M2 channel blocking drugs resistant variants were detected in seasonal influenza A(H3N2) and pandemic A(H1N1)pdm09 strains, and variants resistant to neuraminidase inhibitors emerged during 2008 in seasonal influenza A(H1N1) after permissive mutations gaining. Molecular characterization of influenza virus allowed the detection of emerging genetic variants, with potential to evade antibodies vaccine and become resistant to antiviral drugs. Moreover, results obtained provide useful laboratory criteria for control and prevention policies update by the Ministry of Public Health, and the future perspective new forms of therapy directed to virus-host interactions.

1417

THE MOLECULAR EPIDEMIOLOGY AND PHYLOGEOGRAPHY OF H3N2 INFLUENZA VIRUS IN PERU

Simon Pollett[1], Martha Nelson[2], Matthew Kasper[1], Yeny Tinoco[1], Mark Simons[1], Candice Romero[1], Marita Silva[1], Edward C. Holmes[3], Daniel G. Bausch[1]

[1]U.S. Naval Medical Research Unit - 6, Lima, Peru, [2]National Institutes of Health, Bethesda, MD, United States, [3]Marie Bashir Institute for Infectious Diseases and Biosecurity, University of Sydney, Sydney, Australia

The evolutionary dynamics of H3N2 influenza viruses in tropical regions like Peru remain unclear, including whether lineages persist in the tropics and seed temperate areas. We aimed to test the 'source-sink' model and clarify the migration patterns of H3N2 within and between Peru and the rest of the world. Respiratory specimens from community-based influenza surveillance cohorts were collected from 2010-2012 in four ecologically diverse sites in Peru: Cusco, Tumbes, Puerto Maldonado (PM) and Lima. H3N2 positive specimens (by QIAamp Viral RNA Isolation Kit assay) were randomly selected over time and space and the complete hemagglutinin (HA) gene sequenced and compared with sequences in GenBank and GISAID databases. Alignment and DNA model selection were performed using MEGA and JmodelTest2 software, respectively. A maximum likelihood (ML) tree of all sequences was inferred using RaxML software. A maximum clade credibility (MCC) tree and time to most common recent ancestor (TMCRA) of Peruvian sequences were inferred using BEAST software, with spatial clustering robustness tested by BaTS software. Of 400 specimens selected , 389 were able to be sequenced. ML analysis of Peruvian and 2023 global comparator sequences demonstrated interseasonal extinction of Peruvian clades. Moderate clustering of Peruvian taxa and mixing with global strains were noted at all study sites. A short TMCRA of Peruvian H3N2 taxa was noted (3.8 years), consistent with rapid replenishment of the Peruvian H3N2 gene pool from international regions. The MCC tree of Peruvian taxa revealed a well-supported spatial structure at all sites ($p < 0.01$), although there was also moderate spatial mixing. Spatial clustering was weakest in Lima and PM (mean maximum clade sizes of 8.04 and 8.2, respectively). In conclusion, there is no evidence of a 'sink-source' dynamic or viral persistence in Peru. Rather, our data supporting a model of ever-migrating global metapopulations of H3N2. Peruvian H3N2 strains are replenished by a well-mixed global gene pool each season with gene flow in and out of the country at multiple locations. While spatially structured, there is evidence of H3N2 migration within Peru, particularly at the Lima and PM sites, which is consistent with high fluxes of human movement and/or larger population sizes at these two locations. These findings have implications for pandemic influenza planning in Latin America and beyond.

1418

CYTOKINE RESPONSE OF RHESUS MACAQUES EXPOSED TO LIVE EBOLA ZAIRE VIRUS CHARACTERIZED WITH MAGPIX PARAMAGNETIC BEAD TECHNOLOGY

Matthew Voorhees

United States Army Medical Research Institute for Infectious Diseases, Frederick, MD, United States

Viral Hemorrhagic Fevers (VHFs) are serious, frequently fatal illnesses characterized by fever and unusual susceptibility to bleeding. VHFs are caused by single-stranded RNA viruses from the families Arenaviridae, Bunyaviridae, Filoviridae, and Flaviviridae. Several aspects of the illnesses contribute to their importance as biological threat agents. Diagnosis is usually made at a reference lab with high risk (BSL3-4) biosafety capabilities. Early diagnosis is critical for proper management of the illness and the prevention of spread. Understanding the pathophysiology of the disease is necessary to be able to develop effective medical countermeasures. To determine if any cytokine responses might convey increased survivability, serum was analyzed from 32 Rhesus macaques that had been exposed to 1, 10, 100, or 1000 pfu of Ebola Zaire virus. Using Luminex's MAGPIX paramagnetic bead platform, the serial bleed live virus samples were analyzed in biocontainment for the development of cytokines important in immune response following infection. Using Life Technologies' Cytokine Monkey Magnetic 29-Plex Panel, time point samples were analyzed from both surviving and non-surviving animals, and has provided data on cytokine responses that correlate with the severity of Ebola virus infection.

1419

RABIES IN IRAQ: 2014 UPDATE

Mashair Z. Ismail[1], Daniel Horton[2], Eman S. Siryan[3], Abdul R. A Wali[2], Husam E. Ab-dullah[2], Emma Wise[2], Katja Voller[2], Graeme Harkess[2], Denise A. Marston[2], Salah F. Abbas[1], Lorraine M. McElhinney[2], Sumaia B. Hassan[4], Anthony Fooks[2]

[1]Central Veterinary Laboratory, Iraq State Company for Veterinary Services, Baghdad, Iraq, [2]Animal Health and Veterinary Laboratories Agency, Weybridge, United Kingdom, [3]Baghdad Veterinary Hospital, Iraq State Company for Veterinary Services, Baghdad, Iraq, [4]Zoonoses Diseases Section, Centers for Disease Control and Prevention, Ministry of Health, Baghdad, Iraq

Rabies control requires the combination of dependable resources, cooperation between veterinary and public health officials and accurate surveillance methods. In prior work we reported trends in human rabies cases between 2001 and 2010 and characterization of animal rabies strains from Baghdad, Iraq. Previously, there had been no systematic surveillance for rabies in animals and no laboratory confirmation of disease or virus strains. Three of 40 animal brains were positive using fluorescent antibody testing and hemi-nested RT-PCR for rabies virus (RABV). Phylogenetic analysis using partial nucleoprotein gene sequences demonstrated that the viruses belonged to a single virus variant and shared a common ancestor with viruses dating back 22 years ago from neighboring countries to the west, north and east of Iraq. These results suggested possible multiple introductions of rabies into the Middle East and regular trans-boundary movement of disease. In the present work, we discuss efforts to improve the surveillance and control of rabies in Iraq over the past several years. In 2012 there were 10 cases of rabies and 12,715 dog bites. In 2013 there were 8 cases of rabies and 15,879 reported cases of dog bites. Although 4000 years have passed since the original disease known as rabies, animals and humans are still dying of this preventable and neglected zoonosis in Iraq.

1420

VALIDATION OF A DUPLEX REAL-TIME RT-PCR ASSAY FOR SIMULTANEOUS DETECTION OF INFLUENZA A AND B VIRUSES

Maria E. Silva, Carlos A. Figueroa, Julia S. Ampuero, Carolina Guevara, Alberto Laguna-Torres, Daniel G. Bausch

Naval Medical research Unit - 6, Bellavista, Peru

Influenza viruses have been the cause of major outbreaks and pandemics with high mortality rates throughout human history. Even though vaccines are available, influenza affects around 15% of planet's population yearly. Disease surveillance is essential for rapid identification of cases, allowing implementation of treatment and control measures. Real-time PCR is a powerful diagnostic technique frequently used in influenza surveillance. We developed a duplex real-time RT-PCR to simultaneously detect influenza A and B viruses to meet the need for rapid and effective diagnostics in an respiratory disease cohort in Peru. A combined set of published primers and probes to detect a highly conserved region of the influenza A virus matrix protein (MP) gene and in-house designed primers and probes to detect the influenza B virus MP gene were optimized for single-step RT-PCR using the ABI7500 Fast real-time PCR system. Two-hundred and sixty eight clinical samples were tested using the newly designed duplex assay. Results were compared with those obtained using single-plex RT-PCR assays for influenza A and B viruses designed by the U.S. Centers for Disease Control and Prevention (CDC). Results from the duplex assay were 97% and 100% consistent with the CDC assay for influenza A and B viruses, respectively. In addition, our duplex assay was able to detect two of three influenza A and B virus co-infections. This assay provides a rapid, accurate, highly sensitive and specific diagnostic test for simultaneous detection of influenza A and B viruses.

1421

BATS IN LYSSA, CORONA AND EBOLA VIRUSES ECOLOGY IN NIGERIA; ONE HEALTH PERSPECTIVE TO INFECTIOUS DISEASE CONTROL

Ignatius C. Nwosuh, Clement Meseko, Benjamin Dogonyaro

National Veterinary Research Institute, Vom, Nigeria

The likelihood of an outbreak of emerging or re-emerging infectious disease is contingent on exposure to known or yet to be described reservoir hosts. In recent years, climate change and habitat alteration increase contact between human and animals in shared environment thereby enhancing interspecies transmission of pathogens. Previous studies have shown evidence of lyssa-viruses in fruit bats in Nigeria. These fruit bats are similar to those observed in other countries where corona and Ebola viruses have been identified. Studies on the risk of human exposure to these bats are critical in protecting public health and animal conservation in the perspective of onehealth. We carried out longitudinal survey of bats including, species identification, their habitats, habits and migration pattern in North Central Nigeria and identified human, climatic, arboreal and ecological factors that are likely to cause exposure to excretions and secretions from these animals by direct field observation. Non-invasive specimens including bat guano and urine were collected for virus detection and isolation by ELISA and culture in mammalian cell lines. Oral interviews and questionnaires survey were also carried out to assess knowledge, attitude and practices with regard to bats. Several species of fruit bats of the order pteropodidae and microchiroptera were found at the forest fringes and within parks and gardens in major cities in North central Nigeria. The choice of habitat is strongly influenced by the forest zones and the presence of forest-like parks and gardens including zoos in the North central. The pattern of migration are also influence by seasonal weather variation such that there is a pattern of movement southward during dry season and northward during rainy season to avoid wetness of the rain and or remain in the south where abundant fruits are available. Bats are reservoir of many emerging and re-emerging pathogens like lyssa-viruses. SARS/MERS corona and Ebola viruses are also considered most likely in these reservoir hosts and because of the interactions of human with bats and games in the forest, game reserve, parks and gardens, the risk of exposure to these pathogens is high. There is therefore an urgent need to design conservation friendly intervention to prevent the pandemics of the future by actions that are taken today.

1422

THE GLOBAL DISTRIBUTION OF CRIMEAN-CONGO HEMORRHAGIC FEVER

Jane P. Messina[1], David Pigott[1], Nick Golding[1], Kirsten Duda[1], John S. Brownstein[2], Peter W. Gething[1], Monica M. Meyers[1], Dylan B. George[3], Simon I. Hay[1]

[1]University of Oxford, Oxford, United Kingdom, [2]Boston Children's Hospital, Boston, MA, United States, [3]Fogarty International Center, Bethesda, MD, United States

Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne infection caused by a virus (CCHFV) from the Bunyaviridae family, and while it occurs primarily in animals, it also occurs in humans who work closely with these animals. Healthcare workers in endemic areas are similarly at high risk. There is no safe and effective vaccine against CCHFV which is widely available, and thus treatment for the potentially fatal disease remains primarily supportive. Therefore, an improved understanding of the distribution and level of risk for CCHF is essential for guiding improvements in disease control strategies. Here we undertake an exhaustive assembly of known records of CCHF occurrence worldwide from 1961 to the present, and use a formal modelling framework to map the global distribution of CCHF risk. We do this by first deriving a consensus on country-level presence or absence, and combine this information with the locations of known occurrences and a suite of high spatial-resolution covariates related to climate, urbanisation, agriculture,

and livestock presence to derive the probability of occurrence at a 5km x 5km resolution globally. We find CCHF to be confined to Africa, Eastern Europe, and western Asia, but with spatially heterogeneous levels of risk within these regions. Our new risk map provides novel insights into the global, regional and national threat posed by CCHF, and highlights the need for cohort studies to be carried out in high-risk zones in order to determine the public health burden posed by this neglected disease. We intend for our contemporary risk map to serve as a starting point for a wider discussion about the global impact of CCHF, and for it to help guide improvements in drug and vector-control strategies as well as evaluation of the economic burden caused by this disease.

1423

CHARACTERIZATION OF PUNTA TORO VIRUS RESPONSIBLE OF HUMAN DENGUE-LIKE CASES IN PANAMA

Davis Beltrán[1], Jean-Paul Carrera[1], Yamilka Díaz[1], Patricia V. Aguilar[2], **Sandra L. Lopez-Verges**[1], Robert B. Tesh[2]

[1]Gorgas Memorial Institute of Health Studies, Panama, Panama, [2]University of Texas Medical Branch, Galveston, TX, United States

The genus Phlebovirus (Bunyaviridae family) comprises over 70 antigenically distinct serotypes of viruses with a wide distribution along the tropics. Punta Toro virus (PTV) is part of the Phlebotomus fever viruses (the sand fly group). In the Americas, PTV has been isolated only in Panama from sand flies, slots, human febriles and sentinel hamsters, and viruses from Punta Toro serogroup have been described also in Colombia and Brazil. Many arboviruses, as PTV, cause in humans symptoms similar to Dengue infection; thus the true number of PTV cases could be underestimated in this Dengue endemic country. Up to a 35% seroprevalence for PTV has been reported in Panama before 1988, however there is no recent data about PTV seroprevalence and about the range of clinical illness caused by this virus. The aim of our study is to evaluate the presence of PTV in human acute sera samples referred by the Dengue surveillance program from 1998 to 2013. We inoculated Vero cells with samples that were Dengue negative, and, for now height samples from patients from Western Panama and Panama city induced cytopathic effect in Vero cells. The isolated virus was characterized as PTV by hemaglutinin inhibition assay. Fragments of the segments L, M and S of the genome of PTV were amplified to perform sanger sequencing and the obtained sequences were aligned and analyzed to compare with previous strains isolated in Panama and PTV serogroup from other regions. The phylogenetic trees show that these strains are related to previously described strain GML902878 (isolated from sentinel Sirian hamster in 1976), and are close to Balliet, a strain related to mild disease in hamster models that was isolated in 1966 also in Western Panama. Our preliminary findings suggest that PTV close to Balliet strain circulates continuously in Western and Central Panama and causes undifferentiated febrile symptoms in humans, underlining the fact that many arboviruses like PTV could be responsible of the less than 30% of Dengue-like cases that are negative for dengue in this country.

1424

COVERAGE DURING AN IMMUNIZATION CAMPAIGN PROVIDING INACTIVATED AND ORAL POLIO VACCINES IN REFUGEE CAMPS AND HOST COMMUNITIES, KENYA - DECEMBER 2013

Fredrick M. Makokha[1], Ahmed Unshur[2], Abdullahi Hussein[1], Gedi Mohamed[3], Wences Arvelo[2], Ian Njeru[4], Samuel Amwayi[1], Samuel Okiror[3], Ondrej Mach[5], Concepcion F. Estivariz[6]

[1]Field Epidemiology and Laboratory Training Program, Nairobi, Kenya, [2]Division of Global Health Protection, Centers for Disease Control and Prevention, Nairobi, Kenya, [3]World Health Organization Country Office, Nairobi, Kenya, [4]Ministry of Health, Nairobi, Kenya, [5]Global Polio Eradication Initiative, World Health Organization, Geneva, Switzerland, [6]Global Immunization Division, Centers for Disease Control and Prevention, Atlanta, GA, United States

Poliomyelitis is a highly infectious viral disease, affecting mainly children <5 years of age. Globally, poliomyelitis cases have decreased by 99% since 1988, but outbreaks continue to occur. In May, 2013 an outbreak of wild type poliovirus with 14 cases was reported in Kenya, among them 13 cases were from Dadaab refugee camps and host communities. An immunization campaign providing inactivated poliovirus vaccine (IPV) and oral polio vaccine (OPV) was launched. We conducted a post-campaign coverage survey to assess the impact and guide future use of IPV. We selected 30 blocks in each of the five refugee camp, and 30 villages in the host communities, by probability proportional to size with replacement. We visited nine households in each block and five households in each village, plus a convenience sample of nomad settlements. Within each household we collected data on all children <5 years on IPV and/or OPV; the youngest child age 6 to 59 months was selected for questions about OPV received through routine immunization. Vaccine coverage and 95% confidence intervals were calculated accounting for clustering. We enrolled 1,084 households, including 2,173 children from refugee camps and host communities and 118 from nomad households. Coverage of OPV plus IPV in the December campaign was 92.8 %(90.2%-94.8%) in refugee camps and 95.8%(93.5%-97.3%) in host communities; OPV coverage in the November campaign was 97.2% (95.4%-98.3%) in refugee camps and 97.3%(95.0%-98.5%) in host communities. Among the 118 children <5 years of age from nomadic households, 40(34%) received IPV plus OPV in December, and 37(31%) had received OPV in November. Among caregivers, 1009(99%) reported being aware of the campaign; 766(76%) knew from megaphone announcements, 475(47%) from social mobilizer, 435(43%) from healthcare worker, and 367(36%) heard from the radio. Among 107 children >6 weeks old who missed IPV, 49(46%) caregivers cited not knowing the location of the vaccination. IPV was successfully delivered with high community acceptance both in refugee camps and host communities. Strategies are needed to improve coverage in nomadic populations.

1425

HOW UNPREPAREDNESS FOR AN EBOLA OUTBREAK LEADS TO A WIDESPREAD EPIDEMIC AND COMPLEXITY TO HALT THE EPIDEMIC; AN ANALYSIS AND DESCRIPTION OF ENCOUNTERED OPERATIONAL CHALLENGES DURING A MÉDECINS SANS FRONTIÈRES INTERVENTION IN THE REPUBLIC OF GUINEA AND LIBERIA

Esther Sterk, Gabriel Alcoba, Annick Antierens

Medecins sans Frontieres, Geneva, Switzerland

Suspected cases of hemorrhagic fever were notified in different locations in the Republic of Guinea before the Ebola Zaire virus was identified and the first ever Filovirus epidemic declared in the country on the 22nd of March 2014. The epidemic rapidly spread further to the capital Conakry and cross border to Liberia. In less than 4 months a cumulative total of 163/25 suspected cases and 112/12 deaths were notified in Guinea and Liberia respectively. The attack rate and case fatality observed

are comparable with previous Ebola outbreaks; however the large geographical spread of the disease is unprecedented and leads to complex operational challenges during the interventions put in place by Médecins sans Frontières in collaboration with the Ministries of Health. The first cases were misdiagnosed because of the unfamiliarity of the disease among health staff. Disease confirmation was hampered by the lack of a reference laboratory in Guinea and challenges around sample transport. Rapid geographical spreading was achieved by the high mobility of cases unaware of their status or looking for better perceived health care. Interhuman contact was not minimized because of lack of knowledge and the non-respect of universal precautions. Corpses were moved to different locations and traditional burials greatly contributed to the spread of the epidemic. Misconceptions in the community resulted in difficulties in accepting isolation of patients and lead community members to attack and chase Médecins sans Frontières team members from an intervention site. Filoviridae can achieve an important geographical spread if countries at risk for outbreaks are not prepared. MSF advocates for awareness of viral hemorrhagic fevers, increasing the level of universal precautions at all levels, training of health staff, national laboratory testing possibilities and assuring emergency preparedness at national level. Research and development should be high on the agenda for the availability of rapid diagnostic tests for viral hemorrhagic fever viridae, active and passive immunizations for infected patients and the passive immunization of communities at risk.

1426

KINETICS OF POLIO SHEDDING FOLLOWING ORAL VACCINATION AS MEASURED BY QRT-PCR VERSUS CULTURE

Mami Taniuchi[1], Sharmin Begum[2], Md Jashim Uddin[2], James A. Platts-Mills[1], Jie Liu[1], Beth D. Kirkpatrick[3], Anwarul H. Chowdhury[4], Kh Mahbuba Jamil[4], Rashidul Haque[2], William A. Petri, Jr.[1], Eric R. Houpt[1]

[1]University of Virginia, Charlottesville, VA, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [3]University of Vermont, Burlington, VT, United States, [4]Institute of Public Health, Dhaka, Bangladesh

Measurements of oral polio vaccine (OPV) shedding in stool are useful to evaluate mucosal immunity, to estimate the burden of vaccine strain poliovirus, and for surveillance post-polio eradication. We developed an one-step serotype-specific real-time RT-PCR for detection of Sabin1, Sabin2, and Sabin3 strains along with an extrinsic internal control, MS2, to normalize the targets for extraction and amplification efficiency. Trivalent OPV (tOPV) was administered at week 6, 10, 14, and 52 weeks (at week 39 half of the infants received IPV and the other half tOPV) in a birth cohort study in the Mirpur region of Dhaka, Bangladesh. This assay was used to intensively study OPV shedding kinetics at weeks 14 (n=88 infants; 42 female and 26 male) and 52 (n=182 infants; 84 female and 98 male) post vaccine administration directly from stool specimens collected before the OPV administration (day 0) and on days +4, +11, +18, and +25 after administration. Of the 1350 samples examined (270 infants × 5 time points), sensitivity and specificity of qPCR was 89% and 91%, respectively, when compared to culture. Overall, the PCR detected more shedding than the standard culturing methods. A quantitative relationship was observed between culture+/qPCR+ specimens and culture-/qPCR+ specimens namely the average burden of shedding in viral copy number from the culture+/qPCR+ specimens was higher than in the culture-/qPCR+ specimens (qPCR copies $3.37\times10^{7}\pm1.22\times10^{7}$ versus $3.88\times10^{5}\pm1.45\times10^{5}$, respectively; Mann-Whitney P<0.001 two-tailed). Kinetics of shedding as revealed by qPCR and culture were generally similar at both time points. A qPCR cutoff of approximately 10^{4} viral copies on day 11 or day 18 post OPV could be used to identify the culture-positive shedders after immunization as well as their shedding duration and intensity. qPCR revealed that S3 (6.4%) was most commonly shed followed by S1 (5.1%) and then S2 (1.9%) , and mixed infections occurred in 6.5%. Our findings suggest that this one-step qRT-PCR polio assay can be used to approximate shedding both qualitatively and quantitatively, and will be useful in monitoring OPV efficacy or transmission during eradication efforts.

1427

MECHANISMS OF VIRULENCE OF MONKEYPOX VIRUS: DELETION OF GENOMIC REGIONS AND THEIR EFFECTS IN PATHOGENESIS

Juan G. Lopera[1], Elizabeth A. Falendysz[2], Tonie E. Rocke[2], Jorge E. Osorio[1]

[1]University of Wisconsin, Madison, WI, United States, [2]National Wildlife Health Center, U.S. Geological Survey, Madison, WI, United States

Monkeypox virus (MPXV) causes a human disease similar to smallpox which is endemic in equatorial Africa. However, the emergence of MPXV in 2003 in the Western Hemisphere (USA) demonstrated the potential of these viruses for geographic expansion and worldwide transmission. Understanding the viral genetic factors associated with virulence are of vital importance for surveillance, prevention and treatment of human MPX disease. Using bioinformatics and molecular virology approaches, we identified and evaluated the effects of deletion of two genomic regions in the highly virulent MPXV-Congo strain. *In vitro* and *in vivo* studies indicated that these genomic regions play a significant role in MPXV replication, tissue spread, pathology, and mortality in susceptible CAST/EiJ mice. In this study, we demonstrated that deletion of multiple immunomodulatory (IMM) genes in MPXV is necessary to produce a pronounced attenuating effect, suggesting that targeted genomic regions contain more than one major MPXV virulence factor. More importantly, we observed marked attenuation of virus with simultaneous deletion of two regions (MPXV-ΔR1/R2) which illustrates the additive effect of genomic regions in MPXV pathogenicity. Deletions of MPXV regions in the highly virulent MPXV/Congo genome hindered cell culture growth and significantly reduced morbidity, replication, spread and mortality in infected CAST/EiJ mice. Thus, parental MPX-Congo/Luc+ caused 100% mortality while all mice infected with recombinant MPXVs/Luc+ with deletions of genomic regions survived to infection and did not show clinical signs of disease. Further, serological and histopathological evaluation confirmed that deletion of genomic regions reduced MPXV tissue pathology and elicited strong antibody responses. Our results support the hypothesis that MPXV pathogenesis is not determined by a single gene. Rather, it is the result of the combined effects and interactions of multiple viral and host factors.

1428

REPORT OF A PROSPECTIVE STUDY IN MENINGOENCEPHALITIS IN PERU

Nicanor Mori[1], Joseph R. Zunt[2], Violeta Celis[3], Eduardo Ticona[4], Grimaldo Ramirez[5], Moises Sihuincha[6], Marizabel Rozas[7], Martin Tipismana[8], Matthew Kasper[9], Drake H. Tilley[10], Thanh Ton[2], **Silvia M. Montano**[10]

[1]Hospital Daniel Alcides Carrion, Callao, Peru, [2]University of Washington, Harborview Medical Center, Seattle, WA, United States, [3]Hospital Belen, Trujillo, Peru, [4]Hospital Dos de Mayo, Lima, Peru, [5]Hospital Emergencias Pediatricas, Lima, Peru, [6]Hospital Cesar Garayar Garcia, Iquitos, Peru, [7]Hospital Regional del Cusco, Cusco, Peru, [8]Hospital Cayetano Heredia, Lima, Peru, [9]Division of Global Emerging Infections Surveillance and Response System, Silver Spring, MD, United States, [10]U.S. Naval Medical Research Unit - 6, Callao, Peru

Background: Meningoencephalitis (MEC) is a significant public health problem throughout the world; however, few studies have defined the etiologies outside North America and Europe. The objective of this study was to determine the etiologies of meningoencephalitis in Peru. Methods: We conducted hospital based surveillance at 12 hospitals in five Peruvian cities including Coast, Andean and Jungle geographical areas. Symptoms and medical history was obtained from patients older than 28 days with

suspected viral MEC; serum, CSF, rectal and nasopharyngeal swabs were also collected. Follow-up visits were conducted 14 days after presentation. Samples were tested for HSV-1 and 2, HIV, and 18 other viruses. HSV MEC was defined as confirmed (HSV detected by PCR in CSF) or probable (HSV detected in serum or IgG sero-conversion). Results: We enrolled 911 subjects since February 2009. 522 subjects (57.3%) were male; average age was 25.9 years (range 29 days - 86yrs). 31 patients (3.8%) died; 77 (8.5%) were co-infected with HIV. 112 subjects (12.3%)were infected with herpes simplex virus (91 confirmed, 21 probable). HSV sequence was available for 94 participants, of whom 84 (89.3%) had HSV-1 and 10 (11.7%) had HSV-2. Tuberculous meningitis was confirmed in 2 cases and suspected in 13 cases. Seven participants developed meningitis secondary to coxsackievirus. Ten cases were secondary to bacterial meningitis, seven cases were caused by Epstein-Barr virus, six cases by enterovirus, 19 cases by Cryptococcus neoformans, 2 cases by Treponema pallidum, one case by cytomegalovirus, and one by adenovirus. 26 participants exhibited co-infection. Overall, an infectious cause of MEC was identified in 296 (22.6%) participants. Conclusions: This is an updated report on the etiology of community-acquired meningoencephalitis in Peru. HSV infection remains the most common pathogen identified. Unfortunately, 77.3% of cases remain undiagnosed. Additional molecular studies are being implemented to discover the etiological cause of cases that had no pathogen detected.

1429

EVIDENCE OF INTRA-SUBTYPE, INTER-VACCINE CLADE REASSORTANTS OF H3N2 IN GLOBAL SURVEILLANCE SAMPLES

Irina Maljkovic-Berry[1], Melanie C. Melendrez[1], Anthony Hawksworth[2], Gary Brice[2], Patrick Blair[2], Eric Halsey[3], Maya Williams[3], Stefan Fernandez[4], In-Kyu Yoon[4], Leslie Edwards[5], Robert Kuschner[1], Xiaoxu Lin[1], Stephen J. Thomas[1], Richard G. Jarman[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]U.S. Naval Health Research Center, San Diego, CA, United States, [3]U.S. Naval Medical Research Unit - 6, Lima, Peru, [4]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [5]Department of State, Washington, DC, United States

Inter-species and inter-subtype genetic reassortment (antigenic shift) has played a major role in the evolution of pandemic influenza, generating viruses to which there is little to no immunity in the general population, resulting in severe disease symptoms and global spread (i.e. 1918 pandemic). Antigenic drift, on the other hand, has been suggested as being mainly responsible for the evolution of the more mild seasonal influenza, generating substitutions conferring gradual escape from previously acquired immunity and giving rise to the need of a constantly updated influenza vaccine. Recent evidence suggests, however, that the evolution of seasonal influenza is in addition substantially affected by intra-subtypic reassortment. Here we report identification of H3N2 intra-subtype reassortment variants derived from global influenza surveillance samples collected since 2009. Full genome and segment phylogenetic analyses show that the reassorted variants derive from two slightly divergent (0.1-1.2%) but well defined vaccine clades, A/Victoria/361/2011 and A/Perth/10/2010. In addition, we identified variants with segments originating from different geographical areas. Our results illustrate the ability of H3N2 to reassort segments (i.e. HA, NP, PA, NA and NS) between both geographically and antigenically defined clades. Although intra-subtypic reassortment of H3N2 occurs frequently, appearance of persistent reassorted variants originating from antigenically distinct clusters is a rare event that has previously been shown capable of producing unusually severe seasonal influenza. It thus becomes important to follow whether the A/Victoria/361/2011-A/Perth/10/2010 reassorted variants found in this study have the capacity to become fixed in the population. Intra-subtype reassortment adds yet another layer of complexity as vaccines circulate with wild type diversity potentially altering the trajectory of influenza viral evolution.

1430

THE EFFECT OF IMMUNIZATION ON MEASLES INCIDENCE IN THE DEMOCRATIC REPUBLIC OF CONGO

Reena H. Doshi[1], Calixite Shidi[2], Nicole A. Hoff[1], Jean-Jacques Muyembe[3], Emile Okitolonda[4], Anne W. Rimoin[1]

[1]University of California Los Angeles Fielding School of Public Health, Los Angeles, CA, United States, [2]Ministry of Health, Expanded Programme on Immunization, Kinshasa, Democratic Republic of the Congo, [3]National Institute of Biomedical Research, Kinshasa, Democratic Republic of the Congo, [4]Kinshasa School of Public Health, Kinshasa, Democratic Republic of the Congo

Measles continues to be one of the largest causes of vaccine-preventable disease mortality among children under five, despite the fact that a safe and efficacious vaccine is readily available. While global vaccination coverage has improved tremendously, measles outbreaks persist through sub-Saharan Africa. Since 2010, the Democratic Republic of Congo (DRC) has seen a resurgence of measles outbreaks, mainly attributed to severe deficiencies in Routine Immunization (RI) at the Health Zone level, where only 22% of reported vaccine coverage rates reach higher than 90%. We used available data from the 2011-2012 IDSR system for measles suspected cases counts reported weekly by health zone to investigate the decline in measles incidence post-immunization (by health zone) with one dose of measles containing vaccine (MCV1) with and without the addition of Supplementary Immunization Activities (SIAs) in the provinces of Kasai-Oriental and Equateur. The impact of measles immunization by health zone was modeled using negative binomial regression. At the provincial level, in Kasai-Oriental, the mean incidence was 452.7 per 100,000 in 2011, while the mean incidence declined to 167 per 100,000 in 2012. In Equateur, the mean incidence was 15 per 100,000 in 2011, while the mean incidence increased to 148.7 per 100,000 in 2012, despite a September 2011 SIA. However, multivariate modeling at the health zone level showed that each 1% increase in MCV1 coverage was associated with a .4% decrease in incidence. Furthermore, the lack of an SIA in each health zone was associated with a 3.4% increase in incidence. While the mean yearly incidence of measles did increase in Equateur following an SIA these are provincial level estimates. Differences may be explained partially by the fact that vaccine effects are not immediate and the selective age categories of mass campaigns. Repeated occurrences of large-scale outbreaks in DRC suggest that vaccination coverage rates are grossly overestimated and signify the importance of the re-evaluation of measles virus dynamics and prevention and control strategies.

1431

DISCOVERY, CHARACTERIZATION AND ECOLOGY OF A NOVEL HEPATITIS A-LIKE VIRUS IN WILD OLIVE BABOONS (*PAPIO ANUBIS*), UGANDA

Andrew J. Bennett[1], Samuel D. Sibley[1], Michael Lauck[1], Geoffrey Weny[2], David Hyeroba[3], David H. O'Connor[1], Tony L. Goldberg[1]

[1]University of Wisconsin Madison, Madison, WI, United States, [2]Kibale EcoHealth Project, Kanyawara, Uganda, [3]Jane Goodall Institute, Entebbe, Uganda

Hepatitis A (HAV; family Picornaviridae; genus Hepatovirus) is an RNA virus that causes acute inflammatory disease of the liver in humans and nonhuman primates, and is commonly transmitted through the fecal-oral route. Most often associated with food-borne outbreaks resulting from fecal-contamination, more rarely humans have acquired HAV from the handling of infected non-human primates in captivity. Conversely, recent studies discovering high HAV antibody seroprevalence in wild non-human primates have implicated reverse zoonotic transmission in areas of sub-Saharan Africa where human-nonhuman primate contact and conflict occur frequently. We discovered and characterized by Next-Generation Sequencing (NGS) a novel Simian Hepatitis A-like virus in the blood of a wild olive baboon (*Papio anubis*) in Kibale National Park, Uganda. Furthermore, RT-PCR diagnostics detected viral RNA in the feces of 40% of

baboons sampled at the time of blood collection, suggesting the shedding of potentially infectious viral particles into the environment by wild baboons in western Uganda. Additional screening by field-deployable PCR shows non-random distribution of the virus among individuals and groups. Our results implicate this nonhuman primate as a potential zoonotic source of Hepatitis A-like viruses. This study demonstrates the value of NGS for discovering potential reservoirs of zoonotic pathogens, and supports the supposition that HAV-like viruses circulate naturally in wild nonhuman primates.

1432

VALIDATION OF A MULTIPLEX RT-QPCR ASSAY FOR DETECTION OF INFLUENZA A AND B IN CLINICAL SAMPLES

Pool Marcos[1], Victoria Gutierrez[1], Nancy Rojas[1], Jorge Achata[1], Sila Ruiton[1], Emelda Gallardo[1], Luis Segura[2], Marco Galarza[1], Maribel Huaringa[1]

[1]Instituto Nacional de Salud, Lima, Peru, [2]Universidad Peruana Unión, Lima, Peru

Influenza viruses and their subtypes are common worldwide and produce outbreaks, often along with other respiratory viruses. In Peru, a nationwide surveillance program performs diagnosis and reports to CDC on the outbreak status and emerging influenza variants. Our aim was to develop a multiplex real-time assay for simultaneous detection of Influenza Virus A and B as well as identification of the Influenza Virus A(H3N2) and (H1N1) pdm09 variants as a way to improve the diagnosis speed. The assay consists of two one-step multiplex real-time PCR reactions (RT-qPCR). We discriminated between Influenza A and Influenza B using the matrix gene of Influenza A virus and the nucleoprotein gene of Influenza B virus. We determined subtypes H3N2 and H1N1pdm09 of Influenza A Virus using the hemagglutinin gene. The RT-qPCR reactions were standardized by amplification of serial template dilutions from isolates provided by the CDC. To validate the assay, we analyzed 109 selected clinical samples from patients collected during 2013. Samples were previously analyzed as part of a diagnosis screening and tested positive for influenza viruses. No cross-reaction was recorded with other respiratory viruses potentially present in clinical samples like Adenovirus, Parainfluenza 1, 2 and 3, Human Respiratory Syncytial Virus and Metapneumovirus. No cross-reaction was found either between samples carrying H1N1pdm09 and H3N2. The cutoff was determined at Cq ≤35 for diagnostic test on both reactions. All Influenza A(H3N2), Influenza A(H1N1)pdm09, and Influenza B clinical samples were diagnosed with 100% concordance. The assay was 100% specific for the detection of influenza subtypes in the sample analyzed. This assay is faster and more cost effective than the one reaction per tube setup previously used in the Peruvian surveillance program.

1433

EVALUATION OF RISK FACTORS FOR HIGH RESPONSE TO ROTAVIRUS IGA AT BASELINE

Deok Ryun Kim[1], Ayan Dey[1], Anuradha Sinha[2], Bisakha Haldar[2], Byomkesh Manna[3], Suman Kanungo[2], Ranjan K. Nandy[2], Mohammad Ali[4], William A. Petri[5], Thomas F. Weirzba[1]

[1]International Vaccine Institute, Seoul, Republic of Korea, [2]National Institute of Cholera and Enteric Diseases, Kolkata, India, [3]National Institute of Cholera and Enteric Diseases, Seoul, India, [4]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [5]University of Virginia, Charlottesville, VA, United States

Poor immune responses to rotavirus vaccination were observed in infants in developing countries with sero-protection of roughly 40% after vaccination. The reasons for these lower immune responses are not well understood. Therefore, it is important to measure the preexisting factors which may effects response to vaccine. We evaluated the risk factors for influencing rotavirus IgA in infants living in the urban slums of Kolkata, India. We recruited 372 infants who were 6 weeks of age, and collected their blood samples and mother's breast milk at the time of enrolment. Considering skewed distribution of rotavirus IgA titers, quantile regression that estimates conditional median or other quantiles of the response variable was used to evaluate the risk factors for rotavirus IgA response at 6 weeks. Covariates, such as mother's breastmilk at 6 weeks, mother's height, mother's BMI, and monthly household expenditure (in 1000 INR), a proxy for socio-economic status, were selected for the multivariable model. The 25th quantile regression model yielded that infant rotavirus serum IgA at baseline was significantly influenced by mother's nutritional status, which was also supported by the 50th quantile regression and the ordinary regression methods indicating the relationship is stable. Mother's BMI influenced infant rotavirus IgA titers by an average of 2.5 titers/ 10 unit of BMI in ordinary regression, and 5 titers/10 unit of BMI in 50th quintile regression analysis. However, the 25% quantile regression explained only 1.2 titers per 10 unit of mother BMI. Household socioeconomic status was found significant in lower quartile but not in higher quantile suggesting the relationship with the IgA serum level is not stable. The study identifies mother's nutritional status influence baseline serum level for rotavirus IgA in infants in India, thus this need to be considered while evaluating immunogenicity of the rotavirus vaccines. The results also suggest that the quantile regression is a useful statistical tool as it provides flexiblity to detect trends in skewed data.

1434

RENAL PATHOLOGY IN THE RHESUS MACAQUE/ *PLASMODIUM COATNEYI* MODEL FOR SEVERE MALARIA

Eric Lombardini[1], Imerbsin Rawiwan[1], Peter Weina[2], Arthur Brown[1], Gareth Turner[3]

[1]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]Walter Reed Army Institute of Research, Forest Glen, MD, United States, [3]MORU, Bangkok, Thailand

Severe falciparum malaria in adults often results in a spectrum of renal pathology ranging from minimal tubular degenerative changes to severe tubular epithelial degeneration and necrosis with hemoglobinuria, cellular casts and proteinosis, consistent with acute renal failure. The pathogenesis of disease is theoretically linked to damage to the endothelial damage within the microcirculation, inflammatory mediators, hemodynamic disturbances and hemolysis. *Plasmodium coatneyi* is one of the non-human primate malarias which serves as an animal model for *Plasmodium falciparum* induced disease in humans. We examined and described the renal pathology of 40 retrospective cases of *P. coatneyi* infection in rhesus macaques. Macroscopic evaluation of the samples was conducted by board certified veterinary and medical pathologists and were correlated with available antemortem clinical data to include terminal parasitemias. In these animals there was significant capillary sequestration within the renal interstitium as well as within the glomerular tufts as well as irregular thickening of the glomerular mesangium.. Furthermore, and closely correlating with the degree of parasitemia, there was increasing severity of vacuolar tubular epithelial degeneration and necrosis, intratubular proteinosis, hemoglobin and cellular casts, as well as parasitized erythrocytes, erythrocytic and histiocytic hemozin pigment and interstitial hemorrhage, fibrin and edema. Interestingly, there is relative absence of inflammation within the affected tissues. These findings are for the most part consistent with those described in adult humans diagnosed with malaria associated renal failure (MARF). As a result of the correlation of the antemortem symptomology, clinical and histopathologic findings in these retrospective samples, we demonstrate the utility of this animal model for specific use in the examination of acute renal failure in severe malaria.

1435

INCREASED LEVELS OF S-NITROSYLATION IMPROVES OUTCOMES IN A MODEL OF EXPERIMENTAL CEREBRAL MALARIA

Robyn E. Elphinstone[1], Jonathan S. Stamler[2], Kevin C. Kain[1]

[1]Sandra Rotman Centre for Global Health, University Health Network-Toronto General Hospital, Tropical Disease Unit, Department of Medicine, University of Toronto; Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada, [2]Institute of Transformative Molecular Medicine, Case Western Reserve University, Cleveland, OH, United States

Decreased nitric oxide (NO) bioavailability is associated with disease severity and worse clinical outcomes in malaria infection. Traditionally NO was thought to act primarily through guanylate cyclase and the production of cGMP; however, there is now extensive evidence for NO to function via a post-translational modification, S-nitrosylation. S-nitrosylation of proteins has been shown to regulate a wide variety of cellular signaling processes and aberrant S-nitrosylation may thus contribute to many disease processes. We hypothesize that increasing bioavailable NO via S-nitrosylating strategies will improve clinical outcome in malaria. In order to test this hypothesis we examined experimental cerebral malaria (ECM) infection in S-nitrosoglutathione reductase (GSNOR) knockout C57BL/6 mice. GSNOR is an enzyme that reduces S-nitrosoglutathione and, therefore, reduces the amount of S-nitrosothiol (SNO), including S-nitrosylated proteins. The deletion of this enzyme results in increased levels of SNO, thereby increasing NO bioactivity in hematopoietic, endothelial and other host compartments. In the ECM model we infected GSNOR knockout mice or their wild type counterparts with 10^6 red blood cells infected with *Plasmodium berghei* ANKA (PbA). In ECM, mice with deletion of the GSNOR enzyme had significantly improved survival compared to wild type control mice ($p<0.0001$), despite significantly increased parasitemia ($p<0.0001$). The prolonged survival in GSNOR null animals was accompanied by improved Rapid Murine Coma and Behavioural Scores (RMCBS) compared to controls. We are currently investigating the effects of the GSNOR deletion on markers of endothelial dysfunction and blood brain barrier integrity during infection. Moreover utilizing bone marrow transplantation strategies, we are determining whether protection is dependent upon S-nitrosylation of hemoglobin, regulators of endothelial WPB exocytosis or other non-hematopoietic compartments. These experiments will help define whether interventions to increase NO bioavailability through SNOs improves outcome in severe malaria.

1436

CHARACTERIZATION OF *PLASMODIUM VIVAX* BLOOD TRANSMISSION STAGES

Nicanor III Obaldia[1], William Otero[2], Jose E. Calzada[3], Pierre-Yves Mantel[1], Manoj Duraisingh[1], Dyann Wirth[1], Matthias Marti[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Antimalarial Drugs and Vaccines Evaluation Center, Tropical Medicine Research, Panama City, Panama, [3]Instituto Conmemorativo Gorgas de Estudios de la Salud, Panama City, Panama

Development of new tools for the detection of *Plasmodium vivax* sub-patent gametocytemia or asymptomatic carriage in semi-immune individuals is fundamental for the eradication agenda. However, *P. vivax* gametocytes are poorly characterized and only a few markers known. In this study we develop new tools for the characterization and field detection of *P. vivax* gametocytes. Using a down-selection based on orthology to *P. falciparum* gametocyte markers we selected a series of putative *P. vivax* gametocyte markers for antibody production and generation of qRT-PCR primers. Epitope-specific rabbit polyclonal antibodies and exon-exon spanning primer sets were tested in samples from *P. vivax* infected Aotus monkeys. Exon-Exon primers against two putative late stage gametocyte markers, PVX_117730 and PVX_117900, were successfully optimized using synthetic cDNA probes and validated in the Aotus monkey model. In these samples typical *P. vivax* gametocyte morphology, including macrogametocytes and exflagellating microgametocytes, were identified on Giemsa stained smears. Indeed, comparison with Pvs25 demonstrated stage specificity and similar sensitivity as this gold standard for the PVX_117900 primer set. Marker gene expression was detected in infected blood samples directly collected from the animals, or after prolonged *ex vivo* culture. Importantly, indirect immunofluorescence (IFA) assays with PVX_117900 antibodies, labeled *P. vivax* parasites showing gametocyte morphology in spots obtained from Percoll gradient bands, though at low frequency. qRT-PCR analysis of longitudinal sampling upon experimental *P. vivax* infection in Aotus supported previous field observations that asexual parasitemia is positively correlated with gametocytemia. Experiments are underway to apply the qRT-PCR and antibody assays to investigate P.vivax transmission during human infection and to perform histological studies in the monkey model. This work represents an excellent starting point for further characterization of *P. vivax* gametocytes *in vitro* and during infection.

1437

HOST CONTROL OF PARASITE GROWTH IN *PLASMODIUM BERGHEI* INFECTION

Miles P. Davenport[1], Shannon E. Best[2], Ismail Sebina[2], Chelsea L. Edwards[2], Kylie James[2], Deborah Cromer[1], Ashraful Haque[2], David Khoury[1]

[1]The University of New South Wales, Sydney, Australia, [2]QIMR Berghofer Medical Research Institute, Brisbane, Australia

Early infection with *Plasmodium berghei* leads to rapid parasite growth, which slows around day 5-6 of infection. Although splenic clearance is thought to be an important factor in host control of parasite growth, few studies have directly measured parasite clearance. We developed a novel protocol to study the clearance of *P. berghei* infected red blood cells (RBC) *in vivo*. Fluorescently labelled RBCs infected with GFP+ *P. berghei* ANKA (PbA-GFP+) were transfused from donor mice into recipient C57BL/6 mice, and their clearance monitored by regular sampling over the subsequent 24 hours. We compared clearance in two groups of recipient mice; one group of naïve mice, and a second group of mice that were infected with PbA-GFP- 5-days prior. Flow cytometric analysis allowed us to distinguish donor vs. host RBC, and donor vs. host parasites. We used modelling to estimate parasite growth and clearance rates in the naïve vs. 5-day-infected animals, and observed faster clearance and reduced growth of donor parasites in the 5-day-infected animals. However, the changes during infection appeared more complex than simply an increase in clearance in the infected animals. Instead, our modelling suggested that there were differences in both the life-stages of parasites recognised and cleared in infected vs. naïve animals, and in the susceptibility of RBC in these animals. We further analysed the clearance data, focusing on clearance of parasites of different life-stages. We found evidence that trophozoites were more highly targeted in 5-day-infected animals, consistent with a shift towards clearance of earlier life stages over the first 5 days of infection. We also analysed the susceptibility of recipient RBC, by comparing the donor RBC (which were the same in the two groups) parasitemia vs. recipient RBC parasitemia. This showed much higher rates of infection of RBC in naïve mice, consistent with a greatly reduced susceptibility of host RBC in 5-day-infected recipients. This approach provides novel insights into the mechanisms of innate control of parasite growth *in vivo*.

1438

NON-INVASIVE MEASURES OF INCREASED INTRACRANIAL PRESSURE IN MALAWIAN CHILDREN WITH CEREBRAL MALARIA

Erin M. Fedak[1], Samuel D. Kampondeni[2], Michael J. Potchen[3], Montfort B. Gushu[2], Ian J. MacCormick[4], Carol A. Rowley[5], Karl B. Seydel[6], Terrie E. Taylor[6]

[1]Nationwide Children's Hospital, Columbus, OH, United States, [2]University of Malawi College of Medicine, Blantyre, Malawi, [3]University of Rochester, Rochester, NY, United States, [4]University of Liverpool, Liverpool, United Kingdom, [5]National Institute of Allergy and Infectious Diseases, Rockville, MD, United States, [6]Michigan State University, East Lansing, MI, United States

Brain swelling seen on magnetic resonance imaging (MRI) is the best clinical predictor of mortality in Malawian children with retinopathy-positive cerebral malaria (CM). Identifying more affordable and feasible non-invasive measures of raised intracranial pressure (ICP) would facilitate recognition of this high risk group, and simplify the conduct of interventional clinical trials. During the malaria season of 2014 (January - June), we carried out serial MRI every 12-24 hours on children with retinopathy positive CM while they were in coma. Two radiologists independently assessed overall brain volume (BV) on an 8-point scale; any discrepancies were resolved by consensus. The BV scores on admission were compared to papilledema (present/absent) and opening pressure at the time of lumbar puncture (mm cerebrospinal fluid). Two non-invasive measures were assessed on admission and at intervals thereafter: optic nerve sheath diameter (ONSD) measured using ultrasound, and pupillometry (NeurOptics). When increased BV was defined as an MRI score of >6, patients with increased BV were more likely to have papilledema than those without increased BV (Fisher's exact, p<0.04). Using the same cut-off, pupillometry, ONSD and opening pressure had AUROCs of 0.35 (95%CI: 0.18-0.53), 0.66 (95%CI: 0.37-0.96), and 0.84 (95%CI: 0.61-1), respectively. Longitudinal analyses of ONSD, pupillometry and BV and case studies in which BV changed significantly over the course of the hospital stay are in progress to determine the natural history of each of the surrogate markers in relation to MRI findings. The findings to date suggest that ultrasound measures of optic nerve sheath diameter, presence of papilledema, and opening pressure are the most useful surrogates for increased BV as determined by MRI.

1439

VITAMIN D INSUFFICIENCY IS COMMON IN UGANDAN CHILDREN AND IS ASSOCIATED WITH SEVERE MALARIA

Sarah Cusick[1], Robert Opoka[2], Troy Lund[1], Chandy John[1], Lynda Polgreen[1]

[1]University of Minnesota, Minneapolis, MN, United States, [2]Makerere University, Kampala, Uganda

Vitamin D plays a role in the immune response to infectious diseases. Activation of the vitamin D receptor in macrophages results in increased production of the anti-microbial peptides cathelidicin and beta defensin, while vitamin D supplementation reduces the inflammatory response and thus severity of influenza infection in animal models. Therefore, we hypothesized that children with severe malaria would have lower concentrations of plasma 25-hydroxy vitamin D (25[OH]D) than healthy children in a malaria-endemic region. To test this, we measured 25(OH)D in plasma by chemiluminescent immunoassay on samples collected from 40 children between the ages of 18 months and 12 years with severe malaria (20 with cerebral malaria, 20 with severe malarial anemia) and 20 healthy community children (CC) in Kampala, Uganda. We found that low plasma 25(OH)D was widespread: 95% of children with severe malaria (38 out of 40) and 80% of CC (16 out of 20) had insufficient vitamin D levels [25(OH)D < 30 ng/mL]. Of note, 20% of children with severe malaria, but no CC, had 25(OH)D levels < 15 ng/ml. Mean plasma 25(OH)D concentrations were significantly lower among children with severe malaria than among CC [mean (se): 21.2 (1.0) vs. 25.3 (1.6) ng/mL, p=0.03]. In addition, after adjusting for weight-for-age z-score (a measure of overall nutritional status), we found that the odds of having severe malaria declined by 9% [OR: 95% CI = 0.91: 0.83, 1.0] for every 1 ng/mL increase in plasma 25(OH)D. In conclusion, we describe for the first time an association between low vitamin D and severe malaria. These preliminary results suggest a possible role for vitamin D in the etiology of severe malaria. Confirmation of the findings of the present study will set the stage for a clinical trial of vitamin D treatment as a preventative or adjunctive therapeutic intervention to decrease the severity of malarial infection.

1440

THE REMODELLING OF NASCENT RETICULOCYTES BY *PLASMODIUM VIVAX* AND ITS PATHOLOGICAL CONSEQUENCES

Bruce Russell[1], Benoit Malleret[1], Wenn Chyau Lee[2], Rou Zhang[1], Rossarin Suwanarusk[3], Yee Ling Lau[2], Francois Nosten[4], Georges Snounou[5], Laurent Renia[3]

[1]National University of Singapore, Singapore, Singapore, [2]University of Malaya, KL, Malaysia, [3]Singapore Immunology Network, Singapore, Singapore, [4]Shoklo Malaria Research Unit, Mae Sod, Thailand, [5]Département de Parasitologie, Hôpital Pitié-Salpêtrière, Paris, France

The pathobiology of *Plasmodium vivax* infections is poorly understood. As malaria parasite red cell tropism defines the course of invasion and the pathology of the resultant disease, we have focused our efforts to determine the fine specificity of *P. vivax* invasion. To do this, we used a novel field flow cytometry approach to sample the different subsets of infected reticulocytes from vivax malaria patients and a range of *ex vivo* assays. Thus, we were able to determine the fine scale tropism of *Plasmodium vivax* for nascent reticulocytes (Heilmeyer Classes I to III) .Importantly nascent reticulocytes are rare in the peripheral blood, suggesting a cryptic role for bone marrow where such target cells are abundant.Subsequent *ex vivo* culture studies of *P. vivax* (with multiple rounds of maturation and invasion) allowed us demonstrate rapid modification of membrane structure and cytoplasm of the nascent reticulocyte. The shear modulus, immunophenotype and nanostructure of the infected reticulocyte membrane were significantly altered within 3 hours of invasion. We also employed microfluidic and micropipette aspiration methods to investigate the biomechanical implications of *P. vivax* development in the reticulocytes.One key finding of these studies was that *P.vivax* rosetting (a process we recently determined is mediated by reticulocyte glycophorin C) may play a significant role in the in disappearance of vivax schizont from circulation. Interestingly, the rate of *P. vivax* rosetting is clearly affected by certain antimalarial treatments.

1441

RESPONSE OF *PLASMODIUM FALCIPARUM* TO OXIDATIVE STRESS

Anthonia M. Hananiya[1], Vasiliki Pappa[1], Kris Subramanaim[1], Rebecca Greenstein[1], Kirk Dietsch[2], Johan Ankarlev[3], Laura Kirkman[3], Johanna Daily[1]

[1]Albert Einstein College of Medicine, Bronx, NY, United States, [2]Weill Cornell Medical College, New York, NY, United States, [3]Weil College of Medicine, New York, NY, United States

Malaria remains a major cause of morbidity and mortality around the world. Severe malaria and malaria-related mortality are due to the human malaria parasite *Plasmodium falciparum*. Host response to the parasite involves the production of reactive oxygen species (ROS). Malaria encodes antioxidant enzymes, however a full understanding of the parasite response to ROS is lacking. ROS could also induce genetic changes in the parasite that could be beneficial for parasite survival. We first examined transcriptional change in the parasites after 4 hour ROS and identified upregulation of stress response pathways including Response to Heat (GO.

0009408), DNA Repair (GO.0006281) and (GO.0006281 DNA repair). We set out to characterize the parasite response to ROS and determine if *P. falciparum* can adapt to increased levels of ROS. To examine the effect of ROS on growth we cultivated the 3D7 strain of *P. falciparum in vitro* in human erythrocytes at 4% hematocrit in supplemented RPMI media with and without ROS. All experiments began at ~2% parasitemia. Parasite growth curves were determined by microscopy of daily smears. Oxidative stress in the form of continuous extracellular generation of hydrogen peroxide was provided by supplementing the culture with 1 mM Xanthine and increasing concentrations XO. 100 U/ml of superoxide dismutase (SOD) was added throughout to enhance formation of hydrogen peroxide from superoxide radical anions generated by the XO-catalyzed oxidation of X. We identified the lethal dose of XO and determined if the parasite could adapt to sub-lethal concentrations of XO. Parasites treated with sub-lethal concentrations of XO demonstrated a decrease in parasite growth from day 3 to day 4. On day 6 of treatment we observed that the treated parasites demonstrated similar growth as compared to untreated control. Our data suggests that parasites exposed to varying concentrations of exogenous ROS showed decrease in growth, however they were able to adapt and grow normally after a few days. We will examine these adapted parasites genetically through transcriptional analysis to characterize their ROS adaptation and examine if genetic rearrangement occurs. Taken together this work explores the impact of host physiology on the biology of the parasite to inform severe disease models of pathogenesis.

1442

POPULATION GENETIC STRUCTURE OF THE ZOONOTIC MALARIA PARASITE *PLASMODIUM KNOWLESI* IN MALAYSIA

Paul Divis[1], Balbir Singh[2], Fread Anderios[3], Shamilah Hasim[4], Asmad Matusop[5], Samuel Assefa[1], Craig Duffy[1], David Conway[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]UNIMAS, Kuching, Malaysia, [3]Sabah Public Health Department, Kota Kinabalu, Malaysia, [4]Institute for Medical Research, Kuala Lumpur, Malaysia, [5]Sarawak Health Department, Kuching, Malaysia

Human cases of *Plasmodium knowlesi* malaria have been seen in many parts of Southeast Asia, with the largest number in Malaysia, following the first major focus of infections reported from Malaysian Borneo 10 years ago. This parasite is recognized as being zoonotic with wild long-tailed and pig-tailed macaques incriminated as the main reservoir hosts. In order to explore the previous and current transmission of infections, and address whether it may have been adapting to humans recently, it is important to study the population genetic structure of the parasite. In this study, a set of 10 microsatellite loci with tri-nucleotide repeats in the reference *P. knowlesi* genome were developed and validated for genotyping of natural inections by hemi-nested PCR assays. Using these markers, we analysed more than 300 *P. knowlesi* isolates from patients at 9 different sampling sites in Sarawak and Sabah states of Malaysian Borneo and mainland Peninsular Malaysia. All loci were polymorphic in all sampling sites, with more than 100 alleles in total scored across all 10 microsatellite loci, but most individual human isolates had single genotype infections. The mean genetic diversity across the loci was moderate to high, with no significant difference between different sampling sites (He values between 0.65 and 0.75). Levels of multi-locus linkage disequilibrium were very low in all sampling sites, indicating that recombination commonly occurs between different parasite genotypes in mosquito vectors, many of which presumably feed on macaques with multiple genotype infections. Pairwise genetic differentiation was more marked between sites in Borneo and mainland Peninsular Malaysia (FST > 0.10) as compared to differences among sites within Borneo. The human *P. knowlesi* genotype data are compared to isolates of wild macaques in Sarawak to test if there is any restriction in gene flow between the different hosts. These results are being used to strategically plan sampling from selected sites for whole genome sequence analysis in order to scan for possible evidence of host adaptation, and to study recombination in more detail.

1443

DIVERSITY OF ERYTHROCYTE INVASION PATHWAYS USED BY *PLASMODIUM FALCIPARUM* IN AREAS OF CONTRASTING INFECTION ENDEMICITY IN WEST AFRICA

Paul Bowyer[1], Lindsay Stewart[1], Harvey Aspeling-Jones[1], Henrietta Mensah-Brown[2], Ambroise Ahouidi[3], Alfred Amambua-Ngwa[4], Gordon Awandare[2], **David Conway**[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]University of Ghana, Legon, Ghana, [3]Universite Cheikh Anta Diop, Dakar, Senegal, [4]MRC Unit, Fajara, Gambia

Plasmodium falciparum uses a variety of alternative ligand-receptor interactions in order to invade red blood cells. The diversity of these pathways has traditionally been investigated by assessing the ability of parasite isolates to invade red blood cells that have been enzyme treated to selectively remove receptors. To date a variety of assay formats have been reported in different studies, but a standardised assay has not been applied to compare across population samples from diverse locations. Here we investigate *P. falciparum* invasion phenotypes from clinical isolates sampled in three sites on a gradient of transmission intensity in West Africa, using a single assay format. This is the first large-scale comparative analysis of erythrocyte invasion by clinical isolates from different endemic countries assayed in a single laboratory. Assays were performed on over 100 *P. falciparum* isolates from Ghana, Guinea and Senegal, that were cryopreserved at source and thawed so that the laboratory operator of the invasion assay was blinded to the sample source. These isolates were phenotyped for their ability to invade erythrocytes treated with neuraminidase, trypsin, chymotrypsin or a combination of these enzymes, in the first round of invasion following thawing but prior to adaption to culture. RNA was isolated for qRT-PCR from the schizont stage of a subset of these *ex vivo* cultured isolates in order to determine the relative expression levels of parasite invasion ligand genes. The data are analysed to explore the hypothesis that particular invasion pathways are selected in areas of high infection endemicity where there is strong acquired immunity against the parasite ligands, compared with areas of lower endemicity where immune selection is weaker.

1444

IRON DEFICIENCY ANEMIA AND *PLASMODIUM FALCIPARUM* GAMETOCYTOGENESIS

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

[1]Harvard School of Public Health, Boston, MA, United States, [2]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States

Iron deficiency anemia and malaria are overlapping public health concerns in large parts of the developing world. Clinical and epidemiological studies have revealed that iron deficiency is protective against malaria infection in children and pregnant women. Our comparison of *Plasmodium falciparum* growth in iron-deficient and iron-replete RBCs *in vitro* has revealed that *P. falciparum* erythrocytic stage infection is attenuated in iron-deficient RBCs. We hypothesized that the inhospitable environment of iron-deficient RBCs, which inhibits asexual erythrocytic stage *P. falciparum* propagation, may additionally impact the rate and magnitude of *P. falciparum* gametocytogenesis. Here we report the results of our study of (i) the time to and (ii) the degree of *P. falciparum* gametocytogenesis in iron-deficient as compared to iron-replete RBCs *in vitro*. Our study of iron-deficiency and *P. falciparum* provides an invaluable model for studying *P. falciparum* pathogenesis and transmission.

1445

EXAMINING SELECTION ON *PLASMODIUM FALCIPARUM* AT DIFFERENT ENDEMIC SITES WITHIN GHANA

Craig Duffy[1], **Samuel Assefa**[1], James Abugri[2], Nicholas Amoako[3], Thomas Ayorigiya[4], Bronwyn MacInnis[5], Dominic Kwiatkowski[5], David Conway[1], Gordon Awandare[2]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]University of Ghana, Legon, Ghana, [3]Kintampo Health Research Centre, Kintampo, Ghana, [4]Navrongo Health Research Centre, Navrongo, Ghana, [5]Wellcome Trust Sanger Institute, Cambridge, United Kingdom

Populations of the human malaria parasite *Plasmodium falciparum* in West Africa are highly polymorphic while being closely related due to relatively unrestricted gene flow within the region. However, selection on individual local populations may vary significantly due to differences in transmission seasonality, drug pressure and levels of acquired immunity. Adaptation of populations will be a balance between local selective pressures and gene flow from neighbouring regions, which may quickly erode signatures of local selection. This will occur most extensively when populations are separated by only short distances, such as within a single country. Population specific selection has been demonstrated to differ between countries within West Africa, but the subtle differences that may exist between populations within a single country have not been investigated. In this study, the genomes of 101 *P. falciparum* clinical isolates from two different Ghanaian sites (Kintampo and Navrongo) separated by ~350km were sequenced and analysed. Transmission in Kintampo, in the forested centre of the country, is high throughout the year, while Navrongo, near the northern border with Burkina Faso, experiences high but seasonal transmission. Scans for evidence of directional selection identified several signatures apparently unique to Ghana, in that they were not seen previously in other West African countries. Patterns of balancing selection were similar in the two Ghanaian populations with high Tajima's D scores observed at loci expected to be exposed to host immune responses. Comparative analysis of the two populations indicated a very close relationship, with a mean FST of ~0.01 and only a small minority of SNPs with FST > 0.1, indicating few loci that may be under divergent selection and which will be discussed.

1446

DEVELOPMENT AND VALIDATION OF AN *IN VITRO*, CELL-FREE METHOD OF CULTURING MOSQUITO-STAGE *PLASMODIUM FALCIPARUM*

Tatyana Savransky, Lindsey S. Garver, Jack L. Williams, Silas A. Davidson

Walter Reed Army Institute of Research, Silver Spring, MD, United States

Between ingestion of gametocytes by an *Anopheles* mosquito and deposition of sporozoites by that mosquito into the skin of a human host, *Plasmodium falciparum* parasites transition through multiple life-cycle stages. These developmental stages are uniquely present in the mosquito vector and present a plethora of molecular and mechanistic targets for interruption of malaria transmission. However, it can be technically difficult and/or laborious to study these developmental stages and the targets they present because they rely on the mosquito for stage progression and growth. We have developed a cell-free method for culturing mosquito-stage *P. falciparum* (NF54 strain) *in vitro*. By seeding with gametocytes from blood-stage cultures, this proprietary method is capable of producing viable ookinetes, oocysts and sporozoites that maintain GFP expression and exclude trypan blue. Immunofluorescent staining with mAb against circumsporozoite protein (CSP) indicates the sporozoites obtained through this method uniformly express CSP while a liver stage development assay indicates they are able to infect cultured human hepatocytes and progress to liver stage, still expressing GFP. Comparative gene expression and mosquito infectivity assays between culture-derived and mosquito-derived parasites are underway.

1447

COMPARABLE DEVELOPMENTAL AND MORPHOLOGIC STAGES OF *IN VITRO* CULTURED *PLASMODIUM FALCIPARUM* SUPPLEMENTED WITH TWO COMMERCIALLY AVAILABLE SERUM SUBSTITUTES

Jourdan Posner, Sandra P. Chang, William L. Gosnell

University of Hawaii at Manoa, Honolulu, HI, United States

Current culturing techniques for the *in vitro* culture of *Plasmodium falciparum* are well established. Historically, media used for the *in vitro* culture of this organism consisted of RPMI 1640, a classic well-defined basal cell culture medium, and the addition of human serum. The use of human serum as a supplement is an absolute requirement for parasite growth, but is problematic due to biosafety concerns and lot variability. For that reason, ALBUMAX© II, a lipid-rich bovine albumin serum supplement, is now used in parasite culture medium in place of human serum. It's effectiveness as a human serum substitute has been well documented, but its composition is uncharacterized. This is an issue with research investigating parasite metabolomics and proteomics, which require well-defined *in vitro* growth parameters. Recently, MP Biomedicals released a highly purified microbiological grade bovine serum albumin for use in cell culture. The objective of this study was to compare ALBUMAX© II and MP Biomedical's Microbiological Grade BSA as human serum substitutes in *P. falciparum in vitro* culture. Identical culture conditions supplemented with either ALBUMAX© II or MP Biomedical's Microbiological Grade BSA were prepared and ran simultaneously. Short-term cultures analyzed by Giemsa staining and flow cytometry revealed similar growth trends with similar proportions of parasite developmental stages. Ring-stage and trophozoite survival assays were performed to examine merozoite invasion of erythrocytes and their subsequent development. Long-term cultures with either human serum substitute were also maintained to verify similar growth trends.

1448

CO-LOCALIZATION OF PFCSA-L AND VAR2CSA ON SURFACE KNOBS OF *PLASMODIUM FALCIPARUM*-INFECTED ERYTHROCYTES THAT BIND THE PLACENTAL RECEPTOR CSA

Harold T. Obiakor[1], Patricia A. Gonzales Hurtado[1], Sanjay Desai[2], Michal Fried[1], Patrick E. Duffy[1]

[1]Laboratory of Malaria Immunology and Vaccinology/National Institute of Allergy and Infectious Diseases/National Institutes of Health, Rockville, MD, United States, [2]Laboratory of Malaria and Vector Research/National Institute of Allergy and Infectious Diseases/National Institutes of Health, Rockville, MD, United States

Placental malaria (PM) is a major cause of disease in pregnant women and their infants; it results from sequestration of *Plasmodium falciparum*-infected erythrocytes (*Pf*-IE) in the placenta via specific binding to chondroitin sulfate A (CSA). Over successive pregnancies, women become resistant to PM as they acquire antibodies against the novel protein PfCSA-L and the variant surface antigen VAR2CSA, *Pf*-IE surface proteins that bind to CSA. Here, we describe the association of PfCSA-L with VAR2CSA, and provide evidence that both protein exist in complexes at the *Pf*-IE surface. In earlier studies, we reported that PfCSA-L binds with high affinity (K_D = 6.6 x 10^{-9} M) to human placental CSPG by Surface Plasmon Resonance (SPR). We also reported that VAR2CSA and PfCSA-L interact on *Pf*-IE surface knobs (by DuoLink analysis), and that the DBL2X domain of VAR2CSA binds to PfCSA-L with subnanomolar affinity (K_D = 8.6 x 10^{-10} M). Here, we report that immune blots using PfCSA-L monoclonal antibodies detect only the PEXEL cleaved form of PfCSA-L (PfCSA-L_{PC}) in *Pf*-IE membrane preparations. Urea extraction of *Pf*-IE membranes suggests that both VAR2CSA and PfCSA-L are anchored by protein-protein (rather than protein-lipid) interactions on the IE surface, suggesting that they exist in complexes. However, VAR2CSA is resistant to alkaline sodium carbonate extraction while PfCSA-L is mostly extractible, indicative of integral and peripheral membrane proteins, respectively.

Preliminary analysis of co-immunoprecipitation and proteomics analysis confirmed direct association of PfCSA-L and VAR2CSA on the surface knobs of *Pf*-IE. These findings suggest that PfCSA-L interacts with VAR2CSA on surface knobs of *Pf*-IE, where they contribute to the CSA-binding phenotype. We are attempting to immunize rats with recombinant PfCSA-L and VAR2CSA DBL2X complexes to generate antibodies against neo-epitopes that may be capable of blocking *Pf*-IE binding to CSA. As a highly conserved protein of small size (~25 kDa), PfCSA-L appears to be a valuable component of a placental malaria vaccine.

1449

PLASMODIUM FALCIPARUM TOPOISOMERASE II

Devaraja Mudeppa, Shiva Kumar, Sreekanth Kokkonda, John White, Pradipsinh Rathod

University of Washington, Seattle, WA, United States

Type II topoisomerases, which are well-studied drug targets for many infectious agents and cancer, remain poorly understood in the human malaria parasite *Plasmodium falciparum*. Conventional efforts to express this enzyme have been challenging, as with many important malaria proteins. Here we report expression of full-length *Plasmodium falciparum* topoisomerase II (PfTopoII) in a cell-free wheat-germ protein expression system. Electrophoresis of *in vitro* expressed, radiolabeled PfTopoII pointed to a single 169 kDa entity on an autoradiogram. Soluble PfTopoII from translated lysates displayed a magnesium-dependent, ATP-dependent, and salt-sensitive supercoiled plasmid relaxation activity, and also DNA decatenation activity. A partially truncated PfTopoII construct retained full TopoII function and was more stable. PfTopoII was purified on a DNA affinity column, and a simple and sensitive fluorescence-based screen was established to conveniently track PfTopoII decatenation reactions. Preliminary work with existing TopoII inhibitors pointed to selective inhibition of PfTopoII compared to human TopoII. The availability of pure, functional PfTopoII opens up exciting paths to discovery of new classes of antimalarial agents.

1450

THE EBL-1/GLYCOPHORIN B LIGAND-RECEPTOR INTERACTION DEFINES A DOMINANT *PLASMODIUM FALCIPARUM* INVASION PATHWAY

Selasi Dankwa[1], Usheer Kanjee[1], Rays H. Jiang[2], Amy K. Bei[3], Markus Ganter[1], Mischka Moechtar[1], Christof Grüring[1], Ambroise D. Ahouidi[3], Daouda Ndiaye[3], Tandakha N. Dieye[4], Souleymane Mboup[3], Manoj T. Duraisingh[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]University of South Florida, Tampa, FL, United States, [3]Cheikh Anta Diop University, Dakar, Senegal, [4]Aristide Le Dantec Teaching Hospital, Dakar, Senegal

Parasite invasion of red blood cells (RBCs) is an obligatory step in malaria pathogenesis. *Plasmodium falciparum*, the parasite that causes the most virulent form of malaria, has evolved multiple proteins known as invasion ligands that bind to specific RBC receptors to facilitate invasion of human RBCs. The EBA-175/Glycophorin A (GPA) and RH5/Basigin ligand-receptor interactions, referred to as invasion pathways, are under consideration as vaccine targets and have been the subject of intense study to the neglect of others. For this study, we chose to focus on the little-studied EBL-1/Glycophorin B (GPB) invasion pathway because polymorphisms in GPB are prevalent in malaria-endemic regions, suggesting selection from malaria pressure. Through bioinformatic analysis, we have also recently identified considerable variation in GPB transcript levels in individuals from Benin. To elucidate the relative importance of the EBL-1/GPB invasion pathway vis-à-vis the well-described EBA-175/GPA and EBA-140/Glycophorin C (GPC) invasion pathways, we used an *in vitro* RBC culture system to deplete GPA, GPB or GPC via lentiviral transduction of erythroid progenitor cells. We assessed invasion efficiency using a panel of wild type *P. falciparum* lab strains and invasion ligand knockout lines, as well as *P. falciparum* Senegalese clinical isolates and short-term culture-adapted isolates. Our results indicate that the EBL-1/GPB and EBA-175/GPA invasion pathways are of similar and greater importance than the EBA-140/GPC invasion pathway, suggesting a hierarchy of RBC receptor usage in *P. falciparum*.

1451

EFFECTS OF HETEROZYGOUS SICKLE HEMOGLOBIN ON *PLASMODIUM FALCIPARUM* ERYTHROCYTIC GROWTH INDICES *IN VITRO*

Morgan M. Goheen[1], Steve M. Taylor[2], Nirmish Shah[2], Martha A. Clark[1], Carla Cerami[1]

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

Heterozygous hemoglobin S, resulting in sickle cell trait (HbAS), reduces the risk of severe *Plasmodium falciparum* infection in African children by 90%. The precise mechanisms by which HbAS confers this protection from malaria remain poorly understood. Elucidating these mechanisms may enable new strategies to neutralize the parasite therapeutically. Among other impacts of HbAS, it has been reported that parasite growth and invasion in HbAS RBCs is attenuated only at low oxygen tensions (≤5% O2), yet much of this work was done several decades ago with less detailed techniques for evaluating parasite growth and invasion. In effort to further define the phenotype of *P. falciparum* infection in patients with HbAS, we quantified parasite cellular phenotypes while cultivating *in vitro* in erythrocytes containing HbAS or normal adult hemoglobin (HbAA). Specifically, using flow cytometry-based assays, we separately examined the effect of HbAS erythrocytes on overall parasite growth, merozoite invasion of RBCs, and merozoite production (parasite erythrocyte multiplication rate). In addition, we used microscopic analyses to compare the timing of parasite maturation and development in HbAS and HbAA RBCs. With our HbAS versus HbAA RBC invasion analyses, we also report development of a simple two-color invasion assay allowing for direct comparison of parasite invasion into two cell populations labeled with the same fluorophore at differing concentrations. Finally, we investigated the impact of alpha-thalassemia upon the distinct cellular phenotypes in HbAS erythrocytes, because clinical data indicate that the co-inheritance of alpha-thalassemia attenuates the protection against severe malaria conferred by HbAS. These investigations leverage novel tools to refine our understanding of an ancient relationship between parasite and host. Further investigations of this relationship can improve our fundamental understanding of *P. falciparum* pathogenesis and enable the development of strategies to treat and prevent severe malaria.

1452

MICROVASCULAR TISSUE REOXYGENATION IN MALAWIAN CHILDREN WITH CEREBRAL MALARIA

Carol A. Rowley[1], Allison K. Ikeda[1], Karl Seydel[2], Terrie Taylor[2], Hans Ackerman[1]

[1]National Institute of Allergy and Infectious Diseases, Rockville, MD, United States, [2]Michigan State University, East Lansing, MI, United States

Impaired vasodilation and parasitized red blood cell adherence to blood vessel walls is thought to contribute to the pathophysiology of severe malaria, resulting in poor tissue perfusion. Decreased rates of skeletal muscle reoxygenation have been observed in Indonesian adults with severe malaria compared to healthy controls, but studies of tissue perfusion have not previously been performed on children with severe malaria. We measured rates of gastrocnemius-soleus tissue reoxygenation following a 3-minute femoral artery occlusion in Malawian children with cerebral malaria (CM) on each of 3 days following admission and at a 28-day follow-up visit. Children with uncomplicated malaria (UM) were assessed as controls. Children with cerebral malaria had lower maximum reoxygenation rates than uncomplicated malaria patients (median[IQR]; CM admission: 0.75[0.59-1.01] vs. UM: 1.22[1.12-1.34] % O2 saturation/second, p = 0.016). Maximum reoxygenation rate increased on day 3 compared to admission (CM day 2: 0.84[0.63-1.10] % O2 saturation/

second, p = 0.176 vs. CM admission; CM day 3: 1.07[0.98-1.33] % O2 saturation/second, p = 0.005 vs. CM admission). In addition, peak reoxygenation rate increased further at the 28-day follow-up visit (CM Follow-Up: 1.99[0.59-2.74] % O2 saturation/second, p = 0.039 vs. CM admission). Children with cerebral malaria appear to have an acutely diminished capacity to reoxygenate hypoxic tissue.

1453

RETINAL MICROCIRCULATION DYNAMICS DURING AN ACTIVE MALARIAL INFECTION

Emile Gordon[1], Ilker Ersoy[2], Stefan Jaeger[3], Michael Waisberg[4], Mirna Pena[1], George Thoma[3], Sameer Antani[3], Susan K. Pierce[1], **Kannappan Palaniappan**[2]

[1]Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States, [2]Department of Computer Science, University of Missouri-Columbia, Columbia, MO, United States, [3]Lister Hill National Center for Biomedical Communications, U.S. National Library of Medicine, National Institutes of Health, Bethesda, MD, United States, [4]University of Virginia, Department of Pathology, Charlottesville, VA, United States

The development of effective adjunctive therapies to treat cerebral malaria (CM) would have significant clinical impact in Africa where nearly a million children die each year due to CM infections. A better understanding of the cellular and molecular mechanisms underlying CM can lead to improved therapies and vaccines. Microcirculation in the retinal vasculature provides a window to image dynamic changes taking place in the central nervous system during CM disease progression. We have introduced a new video microscopy imaging modality using high resolution fundoscopy (HRF) and optical coherence tomography (OCT) to visualize the course of a *Plasmodium berghei* infection in a murine model of CM. Using OCT measurements the *in vivo* retinal cross-sections of infected mice do not seem to be enlarged or edematous in comparison to uninfected mice. Bright field fundoscopy reveals flowing hyper-reflective clumps that are confined to the retinal vasculature. We are actively investigating the nature of these clumps using fluorescently tagged parasites and flow cytometry to determine whether the size and behavior of the hyper-reflective bodies is correlated with disease severity and parasitemia. Infected mice are easily distinguished from uninfected controls based on the presence of hyper-reflective clumps, which suggests that HRF has diagnostic potential for establishing an individual's infection status. Using fundoscopy we detected a CM-specific increase in the number of GFP-positive immune cells (monocytes, macrophages, granulocytes) in the retina of LysM-GFP mice as the infection progressed. These preliminary data suggest that the retinal microcirculation can serve as a diagnostic window for malarial infection, and using video analysis of the microcirculation dynamics can aid in quantitatively characterizing the development of cerebral malaria under different treatments.

1454

AMPLICON DEEP SEQUENCING OF *PLASMODIUM VIVAX* MEROZOITE SURFACE PROTEIN-1: FROM GENES TO STRAINS TO INDIVIDUALS TO POPULATIONS

Christian M. Parobek[1], Jessica T. Lin[2], Nicholas J. Hathaway[3], Nick Matinyan[4], Deen Garba[4], Mark Janko[5], Oksana Kharabora[2], Suwanna Chaoratanakawee[6], Panita Gosi[6], Charlotte A. Lanteri[6], Chanthap Lon[6], Jeffrey A. Bailey[3], David L. Saunders[6], Jonathan J. Juliano[2]

[1]University of North Carolina at Chapel Hill, Curriculum in Genetics and Molecular Biology, Chapel Hill, NC, United States, [2]University of North Carolina at Chapel Hill, Division of Infectious Diseases, Chapel Hill, NC, United States, [3]University of Massachusetts Medical School, Program in Bioinformatics and Integrative Biology, Worcester, MA, United States, [4]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [5]University of North Carolina at Chapel Hill, School Department of Geography, Chapel Hill, NC, United States, [6]U.S. Army Medical Component, Armed Forces Research Institute of Medical Sciences, Department of Immunology and Medicine, Bangkok, Thailand

Malaria parasites have numerous hypervariable surface antigens. Deep sequencing of genes and the SNPs that encode this variability is a compelling new tool to answer questions about within-host diversity, between-strain competition, population genetics, and the origin of recurrences. Amplicon deep sequencing has been used to investigate these issues in malaria and other organisms. However, several questions regarding this approach remain. First, it is unclear how these results relate to other genotyping methods, such as microsatellites. Second, it is unclear how deep sequencing of individuals correlates to deep sequencing of pooled samples (PoolSeq). Here, we explore these issues while investigating the *Plasmodium vivax* population of Northern and Western Cambodia. Using ion semiconductor sequencing, we sequenced a short hypervariable fragment of the *P. vivax* merozoite surface protein-1 42-kDa domain to investigate the within-host diversity, population diversity and population structure of *P. vivax* using a cohort study (n=108 isolates) and a cross sectional survey (n=159 isolates). In these populations, we identified 67 and 35 unique haplotypes, respectively, and a total of 47 SNPs. Comparing amplicon deep sequencing to a three-locus neutral microsatellite genotyping approach on a subset of 50 isolates, we found that amplicon deep sequencing is more sensitive for resolving and following mixed infections (average MOI of 3.6 vs 2.1 variants per isolate, p<0.001). Direct comparison of PoolSeq to standard individual deep sequencing found that pooled deep sequencing of clinical isolates provides an accurate picture of parasite diversity and captured the great majority (88%) of the diversity within the population. Lastly, different population structures are seen between provinces and within provinces in Northern and Western Cambodia, suggesting that highly structured *P. vivax* populations exist within this region. This study addresses several knowledge gaps concerning the use of amplicon deep sequencing and further demonstrates its utility for studying the genetic epidemiology of malaria.

1455

VARYING INTERACTION BETWEEN *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN AND DUFFY-POSITIVE RED CELLS

Sushma Krishnan[1], Niraj H. Tolia[2], Lenore Carias[1], Brian T. Grimberg[1], Christopher L. King[1], Peter A. Zimmerman[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]Washington University, St. Louis, MO, United States

Plasmodium vivax invasion of erythrocytes is known to be dependent on the interaction between *Plasmodium vivax* Duffy Binding protein region II (PvDBPII) and the Duffy antigen (Fy) present on erythrocytes. However increasing evidences indicates an alternative Fy-independent invasion pathway maybe available. To better understand the mechanisms by which the PvDBP interacts with host erythrocytes we examined binding

of conformationally correct recombinant PvDBPII to Duffy-positive host erythrocytes. We found levels of PvDBPII binding to a single individual's erythrocytes highly reproducible, but we also observed considerable variability in binding among erythrocytes from different individuals of the same Duffy genotype. Some of this inter-individual variation was attributable to the Duffy polymorphism on the N-terminal region of the Fy (Fy^a/Fy^a = 2197.38 ± 608.15 vs Fy^b/Fy^b = 4713 ± 483.16). However, PvDBPII binding between two *FY*B/*B* individuals showed considerable (e.g. individual 1 vs individual 2 = 4713 ± 483.16 v/s 12924 ± 749.79). The results seem to suggest that polymorphisms in other erythrocyte membrane proteins and/or their post-translational modifications could influence interaction between PvDBP and host erythrocytes, thus modifying susceptibility to vivax malaria.

1456

PHARMACOVIGILANCE DURING CAMPAIGN OF SEASONAL MALARIA CHEMOPREVENTION IN SENEGAL, 2013

Ibrahima Diallo, Mamadou L. Diouf, Moustapha Cisse, Alioune B. Gueye, Medoune Ndiop, Mady Ba

Senegal National Malaria Control Program, Dakar, Senegal

In 2013, the Senegal National Malaria Control Program (NMCP) implemented seasonal malaria chemoprevention (SMC), an intervention recommended by the World Health Organization (WHO) in 2012 in areas of seasonal malaria in which at least 60 % of cases occur over a period of four months. In Senegal, SMC was implemented in a door to door campaign by community health volunteers administering a dose of sulfadoxine-pyrimethamine (SP) and amodiaquine (AQ) under directly observed therapy to children three months to ten years and leaving an additional two doses of AQ for the guardian to administer. The campaign took place in four districts with almost 60,000 children in the target age group, and was accompanied by communications, advocacy, and social mobilization activities. While the drugs used in SMC are generally considered safe and effective, they can cause adverse events that may be minor, moderate, or in very rare cases, severe. The implementation of pharmacovigilance of antimalarials was an important step for the development of the pharmacovigilance program in Senegal and helped reorganize the national system, with the appointment of a national focal point at the Directorate of Pharmacies and Laboratories. Senegal has been able to establish a single system that takes into account all medicine programs and other products available in the country. Senegal was the 95th member of the WHO International Drug Monitoring Program and transmits notifications to the Uppsala Monitoring Center via VigiFlow software. During the 2013 SMC campaign, notices of adverse events were collected by the NMCP and processed by the Anti-Poison Center. Of the 115,547 treatments of SP+AQ administered to 59,420 children under 10 years, 20 adverse events notifications were sent to the NMCP. Adverse effects reported were mostly minor: abdominal pain, nausea, vomiting, urticaria, etc. All notifications were made by health post nurses. The Anti-Poison Center, which is responsible for determining imputability, judged that imputability was possible in 18 cases, improbable for one case, and uncategorized for one case, in which a single dose of amodiaquine was given. No severe adverse events were notified. In 2014, SMC will be implemented in 16 districts in the four regions of Kédougou, Tambacounda , Kolda and Sédhiou, targeting nearly 600,000 children. The pharmacovigilance system will be strengthened to ensure that adverse events will be notified and tracked.

1457

SELECTION OF ANTIRETROVIRAL TREATMENT (ART) IMPACTS ANTIMALARIAL PHARMACOKINETICS AND TREATMENT OUTCOMES IN HIV-MALARIA CO-INFECTED CHILDREN IN UGANDA

Sunil Parikh[1], Norah Mwebaza[2], Richard Kajubi[2], Joshua Ssebuliba[2], Sylvia Kiconco[2], Liusheng Huang[3], Qin Gao[3], Abel Kakuru[2], Jane Achan[2], Francesca Aweeka[3]

[1]Yale School of Public Health, New Haven, CT, United States, [2]Makerere University, Kampala, Uganda, [3]University of California San Francisco, San Francisco, CA, United States

HIV-infected children on protease-inhibitor (PI)-based ART have a lower risk for malaria compared to those on NNRTI-based ART. We evaluated the pharmacokinetics (PK) and pharmacodynamics (PD) of artemether-lumefantrine (AR-LR) in Ugandan children aged 0.5 to 8 years, providing the first intensive PK/PD data in HIV-infected children receiving lopinavir/ritonavir (LPV/r) or NNRTI [nevirapine (NVP) or efavirenz (EFV)]. HIV-uninfected children served as controls. Intensive PK (area under the concentration-time curve, AUC) and PD for 28 and 42 days, respectively was done. AR, active dihydroartemisinin (DHA), and LR in capillary plasma were measured by LCMSMS for 121 children (n=30 LPV/r; 28 NVP; 15 EFV and 48 controls). Lower AR AUC was seen with all ART groups compared with controls [geometric mean (GM) ratio; LPV/r 0.79 (ns); NVP 0.36 ($p<0.001$); EFV: 0.42, ($p=0.003$)] while DHA was reduced only in children on EFV [GM ratio 0.27, ($p<0.001$)]. For LR, AUC was 2-fold higher for children on LPV/r and 3-fold lower for children on EFV-based ART ($p<0.001$ for both). Median Day 7 LR level was 3.4-fold higher and 3.9-fold lower with LPV/r and EFV, respectively. Cumulative 28 day risk of parasitologic failure was 12%, 27%, and 33% for children on LPV/r, NVP, and EFV, respectively. Multivariate regression indicates altered malaria risk was largely due to distinctions in LR AUC ($p=0.016$). Moreover, day 7 levels were associated with 28 day risk of recurrent parasitemia (hazard ratio 0.60, $p=0.001$). Notably, 14/15 children on EFV had day 7 levels in the lowest quartile, and 13/15 had LR AUC in the lowest quartile. Use of AL in the setting of LPV/r-based ART resulted in a significant increase in LR exposure, largely explaining a reduced risk of malaria. In contrast, EFV-based ART results in significant reduction in exposure to all drugs; AR, DHA and LR; with LR reduction strongly associated with increased risk of parasitologic failure. These intensive PK/PD data demonstrate altered exposure and response supporting reevaluation of guidelines for antimalarial treatment of HIV-infected children, especially in the setting of EFV-based ART.

1458

ARTEMETHER-LUMEFANTRINE EXPOSURE FOLLOWING TREATMENT IN MALARIA-INFECTED CHILDREN AS COMPARED WITH ADULTS IN UGANDA

Norah Mwebaza[1], Richard Kajubi[1], Liusheng Huang[2], Joshua Ssebuliba[1], Xixi Cheng[2], Sylvia Kiconco[1], Jane Achan[1], Myaing Nyunt[3], Francesca Aweeka[2], **Sunil Parikh**[4]

[1]Makerere University, Kampala, Uganda, [2]University of California San Francisco, San Francisco, CA, United States, [3]University of Maryland, Baltimore, MD, United States, [4]Yale School of Public Health, New Haven, CT, United States

Artemether-lumefantrine (AR-LR) is currently the most widely adopted artemisinin combination therapy world-wide. We evaluated the comparative pharmacokinetics (PK) and pharmacodynamics (PD) of AR-LR in the context of developmental changes occurring in children 6 months to 8 years and compared PK results to data from adults. All individuals were treated for malaria in a high endemic region of Uganda and enrolled for intensive PK evaluations for AR-LR with 42 day follow-up. Exposure was estimated to 21 days [area under the concentration-time curve (AUC)]. Artemether (AR), its active metabolite dihydroartemisinin (DHA), and the long-acting partner drug, LR were quantitated from capillary plasma by LC/

MS/MS. Thus far, intensive PK/PD evaluations have been completed and analyzed for 26 children 8 months to 4 years, 22 children 4 to 8 years, and 11 adults (16 to 56 years) (n=30 enrolled). As expected, parasite densities (parasites/μL) at the time of presentation were significantly different between age ranges [Geometric mean (GM) 20,615, 6232 and 604 parasites/μL in < 4 years, ≥4 to 8 years, and adults, respectively). For the artemisinins (AR and DHA), no significant changes in exposure (maximum concentration or AUC) were observed in children as compared with adults. For LR, a trend toward reduced exposure (AUC) and day 7 (D7) levels were observed for children compared to adults [AUC GM 272 vs 316 hr•ug/mL (p=0.15); D7 median 339 vs 450 ng/mL). Notably, LR levels on day 21 were significantly lower in younger children compared to adults (p=.04). These results suggest overall comparable exposure in children as compared with adults, although sample sizes are limited in the very young (n=8 less than 2 years) and adults (n=11). Enrollment is ongoing in both population and intensive PK studies, and final results will be presented.

1459

THE AFFORDABLE MEDICINES FACILITY-MALARIA (AMFM) IN GHANA: FACTORS ASSOCIATED WITH PRIVATE RETAILER'S ADHERENCE TO THE RECOMMENDED RETAIL PRICE

John H. Amuasi[1], Beth Virnig[1], Graciela Diap[2], The AMFm Independent Evaluation Team[3]

[1]University of Minnesota School of Public Health, Minneapolis, MN, United States, [2]Drugs for Neglected Diseases initiative, Geneva, Switzerland, [3]Komfo Anokye Teaching Hospital, London School of Hygiene & Tropical Medicine, ICF International, Kumasi, Ghana; London, United Kingdom; Rockville, MD, United States

The Affordable Medicines Facility-malaria (AMFm) was initiated as a pilot in 7 countries aimed at increasing availability, reducing prices, increasing market share and increasing use of co-paid quality-assured artemisinin-based combination therapies (QAACTs). As part of the AMFm supporting interventions to facilitate the high level subsidy on QAACTs reaching consumers, the AMFm green leaf-logo was widely publicized, along with a recommended retail price (RRP) in Ghana. Using data from the 2011 endline survey of the Global Fund-commissioned AMFm independent evaluation, we explored factors associated with outlets stocking some co-paid QAACTs at RRP, and those stocking all at RRP in the private-for-profit health sector in Ghana. Analyses accounted for the complex survey design. We used multivariate logistic regressions to determine the association between being aware of the RRP and correctly specifying it, and the probability of stocking some or all QAACTs at RRP. Among the 545 outlets making up our sample, and which stocked at least 1 co-paid QAACT, 1,440 co-paid QAACTs were audited, with a mean number of 2.3 per outlet (95% CI: 2.1, 2.4). Twenty-four percent of outlets stocked no co-paid QAACTs at RRP, while 68% had some, but not all their co-paid QAACTs at RRP. Almost half of all the co-paid QAACTs audited were available at RRP. Many more outlets stocked some co-paid AL over ASAQ (93 % vs. 46%). Knowledge of the RRP was associated with a much higher predicted probability of stocking some co-paid QAACTs at RRP than stocking all at RRP (83% vs. 41%), although it was a strong predictor of both outcomes (p<0.001 for both). The type of co-paid QAACT being stocked (ASAQ/AL/both) was an important predictor of an outlet stocking both some (p=0.014) and all (p=0. 005) co-paid QAACTs at RRP. Malaria prevalence was also associated with stocking some co-paid QAACTs at RRP (p=0.013). Our study shows that retailer's adherence to the RRP for co-paid QAACTs can be high when knowledge about the RRP is present. Information on the AMFm subsidy needs to be disseminated to retailers with greater focus on those areas of high malaria prevalence, such as the northern savanna zone. All recommended policy interventions should be coupled with regular monitoring of prices and other indicators in the market in order to accurately measure the trend of the effects of the interventions.

1460

EFFECTIVENESS AND TREATMENT ADHERENCE TO ARTEMETHER-LUMEFANTRINE UNIT DOSED BLISTER-PACKS VERSUS STANDARD BLISTER-PACKS IN THE TREATMENT OF UNCOMPLICATED MALARIA: A RANDOMIZED CONTROLLED EQUIVALENCE TRIAL

Joaniter Nankabirwa[1], James Tibenderana[2], Samuel Gonahasa[1], Bonnie Wandera[1], Grace Nakanwagi[2], Mark Montague[2], Myers Lugemwa[3], Kirstie Graham[4], Sylvia Meek[4]

[1]Makerere University College of Health Sciences, Kampala, Uganda, [2]Malaria Consortium, Kampala, Uganda, [3]Ministry of Health, Kampala, Uganda, [4]Malaria Consortium, London, United Kingdom

Pre-packing drugs for treatment of uncomplicated malaria, into colour coded unit doses for particular age or weight groups has been shown to improve adherence; however, it creates challenges regarding procurement and administration thus reducing the benefits gained. This study sought to determine whether effectiveness and treatment adherence to standard blister-packs would be equivalent to unit dosed blister-packs. Between February and October 2010, an open label randomised controlled trial was conducted in 846 children aged 6-59 months living in a high malaria transmission setting in Uganda. Enrolled children were randomised to two study arms, receiving either unit dosed or standard blister-packs, and followed for 28 days. Outcome measures were risk of clinical and parasitological failure over 28 days' follow-up and adherence to prescribed treatment. Analyses were conducted on an intention-to-treat basis. The cure rate unadjusted by genotyping was 44.6% in the unit dosed blister-packs treatment arm compared to 41.5% for standard blister-packs (risk difference (RD) 3.1, 95% confidence interval (CI) -3.1, 9.9 p=0.375). Unadjusted risk of clinical failure was 28.7% in both treatment arms and unadjusted risk of parasitological failure was 26.7% and 29.8% in the unit dosed and standard blister-packs arms respectively (RD -3.1, CI -9.2, 3.2, p=0.330). There was no difference in adherence between the two treatment arms. Effectiveness and treatment adherence were equivalent in the two study arms. This study questions the value of unit dosed packaging given the challenges associated with ensuring uninterrupted supply, and highlights the importance of good provider-patient communication for treatment adherence. The findings suggest that standard blister-packs would improve quality of care through improved reliability of supply without compromising effectiveness and adherence to antimalarials. The implications of this study are broader than antimalarials and further research is needed to confirm and explore the potential impact of these findings.

1461

BIOLOGICAL STABILITY OF DIHYDROARTEMISININ IN PHYSIOLOGICAL CONDITIONS

Nicoletta Basilico[1], Silvia Parapini[1], Piero L. Olliaro[2], **Donatella Taramelli**[1]

[1]University of Milan, Milan, Italy, [2]UNICEF/UNDP/World Bank/WHO Special Programme on Research & Training in Tropical Diseases, World Health Organization, Geneva, Switzerland

Artemisinin derivatives are the most effective antimalarials available to-date. Dihydroartemisinin (DHA) is a drug on its own and also the main metabolite of other artemisinins. These molecules, characterized by the presence of an endoperoxide pharmacophore, are highly unstable; they degrade very quickly in the presence of ferrous iron or organic solvents. Less documented is the stability of DHA, measured as antimalarial activity, when incubated *in vitro* with blood components or in different cultures conditions. We investigated this problem by incubating DHA in PBS, plasma, serum or erythrocytes lysate for different lengths of times, at different temperatures and pHs. Chloroquine, a 4-aminoquinoline antimalarial and artesunate were also used to verify if drug instability was related to the presence of the endoperoxide. Residual activity of the drugs was evaluated by determining *Plasmodium falciparum* viability with the

pLDH method. A significant reduction of the antimalarial activity of DHA was seen after incubation in plasma or serum and to a lesser extent with erythrocytes lysate or PBS: 3-hour incubation in plasma was sufficient to double the IC50 of DHA, whereas activity was almost completely lost after 24h. The serum-enriched mediums (10% human serum or 10% albumax) customarily used for *in vitro* cultures also affected DHA efficacy. DHA activity was partially preserved at 4°C or at room temperature, but was lost at 40°C. Similarly, increasing pH from 7.2 to 7.6 reduced DHA efficacy. Artesunate behaved in a similar way to DHA, whereas chloroquine was unaffected in any of the tested *in vitro* conditions. These results suggest that particular care has to be taken in conducting and interpreting *in vitro* studies, and in storing these compounds. Moreover, conditions such as fever, hemolysis or acidosis associated with malaria severity may contribute to artemisinins instability and reduce its effectiveness.

1462

SYNTHESIS AND BIOLOGICAL TESTING OF 2,5-SUBSTITUTED PYRIMIDINES

Victor E. Zottig[1], Stanton Q. Smith[2], Thomas A. Bradshaw[2], Brent S. Clark[2], Zachary A. Ratka[2], Patricia J. Lee[1], Norma E. Roncal[1], David A. Donkin[1], Eric K. Kim[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Virginia Military Institute, Lexington, VA, United States

Heterocyclic containing compounds have historically represented important structures in medicinal chemistry. Malaria is a debilitating and life-threatening mosquito borne disease that affects millions of people a year. From the 1600's to WWII the traditional treatment for malaria used the aromatic heterocylic compound, quinine. Vinamidinium salts are known for their ability to make aromatic heterocycles. We report on the synthesis of pyrimidines from vinamidinium salts and their biological activity against Malaria. We have prepared two vinamidinium salts and then synthesized a series of pyrimidines from each salt. These two series of pyrimidines were evaluated for anti-malarial activity.

1463

MALARIA PLACENTAL INFECTION AND INTERMITTENT PREVENTIVE TREATMENT IN SUBURBAN KINSHASA, DR CONGO

Serge M. Kayembe[1], Fabrice Matuta[1], Aimée K. Kahindo[1], Solange E. Umesumbu[2], Thierry L. Bobanga[1]

[1]University of Kinshasa, Kinshasa, Democratic Republic of the Congo, [2]Malaria National Control Program, Kinshasa, Democratic Republic of the Congo

Malaria is a major health threat and an obstacle in the path of economic development of individuals, communities and nations. It is the leading cause of mortality and morbidity in the DRC. Pregnant women and children under 5 years are the most vulnerable groups. For pregnant women, it may be responsible for premature abortion, fetal growth retardation and infant and maternal death. In the DRC, the NMCP recommends that all pregnant women receive two doses of IPT with sulfadoxin pyrimethamin during ANC. This study was undertaken to 1) determine the proportion of pregnant women who took the IPT, 2) Estimate the frequency of placental malaria infection at N'djili Reference Hospital, and3) Determine the frequency of chorionitis A cross sectional study was conducted among 223 women delivered at the maternity HGR N'DJILI in Kinshasa, women who accepted to participate in the study after informed consent in a period from September 2013 to March 2014. Blood sampling was performed for making a thick and a thin smear in women at childbirth, placenta prints was made and a sample was preserved in formalin for histological analysis. In addition an interview was conducted to obtain information about IPT. 223 women were included in the study. The age group most represented was 18-25 years with 45.4 %. Primiparous were 46.4 %. TPI 1 was observed in 76.9 %, while the taking of IPT 2 was observed at 23.1%. 28.7% of women took the first dose at fifth month and 44.3 % on the sixth month or after . All multiparous took the first dose of IPT, in secondiparous and primiparous group the taking was 66. 6 %. The difference between the two group was highly significant p <0.0001 For the second dose, 68.5% take up to 8th month and 16.6% have taken at nineth month 77.8% of GE examined was positive positive with *Plasmodium falciparum* 73.1% of placental prints were positive with trophozoites and 7.4% with trophozoites and schizonts of *Plasmodium falciparum*. Diiference between women with IPT and women without IPT, was significant p< 0.001 Histopathological findings will be available in late May In conclusion, the plasmodium infection in pregnant women and placental infection are very high among pregnant women in peri -urban environment Kinshasa Intermittent preventive treatment in pregnant women is not unfortunately respected Increased awareness should be held to a greater commitment to the strategy of prevention against malaria

1464

DIRECTLY OBSERVED THERAPY: REVIEW OF BEST PRACTICES AND THE APPLICATION TO MALARIA TREATMENT

Jennifer A. Wegbreit[1], Thang Duc Ngo[2], Anh Quy Nguyen[2], Xa Xuan Nguyen[2], Nicholas Martin[3], Duong Thanh Tran[2], Hien Nguyen[4], Colin Ohrt[1]

[1]University of California San Francisco, San Francisco, CA, United States, [2]National Institute of Malariology, Parasitology and Entomology, Hanoi, Vietnam, [3]Naval Medical Research Center-Asia, Singapore, Singapore, [4]U.S. Embassy, Hanoi, Vietnam

Directly observed therapy (DOT) is the standard of care for tuberculosis treatment and it is used for HIV/AIDS treatment in many settings. These are complex treatment regimens for which high adherence rates have been achieved. Yet, for malaria, where treatment regimens range from three to 14 days, DOT is perceived to be too difficult to implement. We are conducting a literature review of DOT best practices for tuberculosis, malaria and any other applicable disease treatment regimens. We are examining DOT for malaria across a wide variety of treatment protocols. In addition to the literature review, we will interview key informants, including community health workers and village health workers, who have implemented DOT for malaria and other diseases. The aim of the interviews is to better understand the most effective implementation strategies and any challenges encountered. We will examine factors that have promoted and hindered high treatment adherence. The standard interview questionnaire captures structured data from key informants and includes information on DOT implementation strategies, contextual information, treatment regimens, and factors leading to success and failure. It also includes questions about treatment seeking behaviors and access to health services at the community level. The National Malaria Control Program (NMCP) in Vietnam has experience implementing DOT for *Plasmodium falciparum* infection in tier 1 provinces of the containment zone. In 2014-2015, Vietnam's National Institute of Malariology, Parasitology and Entomology (NIMPE), has funding from the Global Fund to implement DOT in areas where multi-drug resistant malaria is emerging. In coordination with NIMPE, we plan to pilot the best practices identified in the literature review and key informant interviews. By June of 2014, we will have completed and analysed 20 structured interviews. The literature review and key informant data will be presented as well as the plan to pilot implementation of DOT.

1465

CHEMOGENETIC PROFILE ANALYSIS OF *PLASMODIUM FALCIPARUM* TO COMPOUNDS FROM THE MMV MALARIA BOX

Kenneth O. Udenze[1], Anupan Pradhan[1], Geoffrey Siwo[2], Zhang Min[1], Asako Tan[2], Naresh Singh[1], Dennis E. Kyle[1], Michael T. Ferdig[2], John H. Adams[1]

[1]Department of Global Health, University of South Florida, Tampa, FL, United States, [2]Department of Biological Sciences, University of Notre Dame, Notre Dame, IN, United States

Drug resistance in *Plasmodium falciparum* requires improved use of existing drugs and enhanced methods for discovery of new drugs with unique targets and mechanisms of action. Chemogenetic profiling of *P. falciparum* mutants is a new approach to identify and prioritize drugs with novel targets and/or modes of action and is potentially a way to predict drug combination therapies with optimal synergistic anti-parasite activity. Isogenic mutants of *P. falciparum* were created by *piggyBac* transposon insertion whereby each mutant parasite carries a unique signature of affected metabolic pathways that can alter responses to drugs. An important advantage of this approach is the precise nature of the chemical-genetic profile, since single mutations are created in an identical genetic background (a clone of NF54). *piggyBac* mutant clones with insertions in identifiable links to specific GO pathways were profiled for responses to a subset of compounds from the malaria box. The wild type and *piggyBac* mutant parasites were allowed to grow for 72 hours in a range of growth inhibitors and then quantified using a DNA dye (SYBRGreen I). The different *piggyBac* mutants varied in their susceptibility to inhibitors, demonstrating unique signatures related to the specific mutation allowing us to map associations among inhibitors and mutants. Cluster and network analyses of chemicogenetic profiles to malaria box drug susceptibility profiles linked drugs with common mechanisms of action and provided potential insights into metabolic pathways targeted by the antimalarial drugs.

1466

OVERCOMING PERSISTENT BARRIERS TO THE SCALE UP OF INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY (IPTP): PERSPECTIVES OF POLICYMAKERS, HEALTHCARE PROVIDERS AND PREGNANT WOMEN

R. Matthew Chico[1], Joyce Wamoyi[2], Daniel Chandramohan[1], John Changalucha[2], Jayne Webster[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]National Institute of Medical Research, Mwanza, United Republic of Tanzania

The World Health Organization recommends intermittent preventive treatment with sulfadoxine-pyrimethamine (IPTp-SP) for pregnant women resident in areas of moderate (stable) or high malaria transmission to prevent the adverse consequences of malaria infection during pregnancy. Despite efforts over the past decade to scale up coverage, less than one-quarter of women receive two doses. To identify persistent barriers to the scale-up of IPTp, as well as the potential to scale-up alternative regimens and/or alternative strategies, semi-structured in-depth interviews (IDIs) and focus group discussions (FGD) were conducted among healthcare providers and pregnant women in Tanzania. A total of 64 pregnant women participated in FGDs, while 28 pregnant women were included in IDIs; 14 healthcare providers participated in IDIs and, separately, 11 policymakers were interviewed. Participant responses were coded and analysed using NVivo 10.0. Content analysis was used to derive a range of themes. A major barrier to the acceptability of IPTp-SP across those interviewed was side-effects. The risk of side-effects discourages some healthcare providers from providing treatment given the ethos of 'do no harm' and the fact that most pregnant women at antenatal presentation are either not infected or have an asymptomatic infection and do not feel ill. Perceptions and experiences of side effects of SP are likely to shape whether or not replacements drugs may be brought to scale given that all current candidates involve multi-day regimens. The risk of side-effects might be more acceptable to many (but not all) policymakers, health care providers, and pregnant women if: a more efficacious therapy than SP is used in IPTp, a replacement for SP is simultaneously protective against malaria and curable sexually transmitted infections, as may be the case with azithromycin-based combination therapies, or women are screened for malaria and only women who are found to be parasitemic are then given treatment.

1467

IN VIVO EFFICACY AND SAFETY OF ARTEMETHER/LUMEFANTRINE VS. DIHYDROARTEMISININ-PIPERAQUINE FOR TREATMENT OF UNCOMPLICATED MALARIA AND ASSESSMENT OF PARASITE GENETIC FACTORS ASSOCIATED WITH PARASITE CLEARANCE OR TREATMENT FAILURE

Deus Ishengoma[1], Celine Mandara[1], Samuel Gesase[1], Esther Ngadaya[2], Sigsbert Mkude[3], Renata Mandike[3], Ally Mohamed[3], Janeth Mgamba[4], Peter Mbuji[4], Ritha Njau[5], Martha Lemnge[1]

[1]National Institute for Medical Research, Tanga, United Republic of Tanzania, [2]National Institute for Medical Research, Dar es Salaam, United Republic of Tanzania, [3]National Malaria Control Program, Dar es Salaam, United Republic of Tanzania, [4]Ministry of Health and Social Wellfare, Dar es Salaam, United Republic of Tanzania, [5]World Heath Organization, Dar es Salaam, United Republic of Tanzania

Antimalarial efficacy studies are recommended by the World Health Organisation to monitor the efficacy of artemisinin based combination therapy (ACT) and possibly detect evolution/emergency of tolerance/resistance to these drugs. Currently, Artemether/Lumefantrine (AL) is the only ACT which is being used in Tanzania and thus, testing of new ACTs such as dihydroartemisinin-piperaquine (DP) is important because alternative drugs are urgently required. This study will be an open-label randomized trial and aims to assess the efficacy of AL versus DP; and the role of parasite genetic/genomic factors that might be associated with treatment outcome among patients with uncomplicated malaria treated with these ACTs. The study will be conducted from May 2014 and will recruit 600 children aged 6 months to 10 years with uncomplicated falciparum malaria at Muheza Designated District Hospital and Ujiji Health Centre in Tanga and Kigoma regions respectively (150 patients per treatment arms at each site). Follow up will be done for 63 days and the primary end point will be parasitological cure on day 28 for AL and 42 for DP (non-adjusted and adjusted by PCR to correct for new infections). The secondary end points will include: parasite clearance after 72 hours, parasitological cure on day 14, extended parasitological cure on day 42 for AL and 63 for DP, improvement in haemoglobin level at day 28 compared to day 0, reduction in gametocyte carriage at day 14 and day 28 Vs 0, occurrence and severity of adverse events, and genomic profile of *P. falciparum* malaria parasite. Preliminary results will be presented and discussed, and study will provide important data to the National Malaria Control Program (NMCP) to be used in the ongoing review of treatment guidelines. The information will also support NMCP to recommend DP as the second line antimalarial drug for the treatment of uncomplicated malaria in Tanzania.

1468

BURIED LEGACY? AN ANALYSIS OF PSYCHIATRIC TOXICITY OF PRE-CHLOROQUINE ANTIMALARIALS

David Adams[1], Daniel Mauck[2]

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

The toxicity of synthetic anti-malarials such as primaquine, chloroquine, and mefloquine have been well documented. Their side effects range from hemolytic anemia in the case of primaquine to psychiatric disturbances in the case of chloroquine and mefloquine. The toxicity profiles of their

pre-WWII predecessors, however, have received relatively little attention. Pamaquine and mepacrine, synthetic anti-malarials developed by German industrial chemists in the 1920s and 1930s, proved invaluable for malaria control among Allied and Axis troops alike. Pamaquine and mepacrine, however, were not without their own toxicity issues. As their use expanded, first in far-flung colonial outposts and subsequently in Asian and European theatres, reports began to emerge about unexpected psychiatric toxicity. Despite these warnings, their central role as anti-malarials continued throughout the Second World War. This presentation will examine the factors that spurred their widespread use despite a growing number of contemporary reports that recommended cautious use among high-risk individuals.

1469

SEVERE MALARIA MORTALITY AND MANAGEMENT IN THREE GENERAL REFERENCE HOSPITALS IN KINSHASA, DEMOCRATIC REPUBLIC OF CONGO

Solange E. Umesumbu[1], Billy S. Kunyu[2], Adonis T. Ntoka[3], Malene B. Masukidi[3], Marilyne L. Mvuata[3], Thierry L. Bobanga[3]

[1]National Malaria Control Program, Kinshasa, Democratic Republic of the Congo, [2]National Institute of Medical Research, Kinshasha, Democratic Republic of the Congo, [3]University of Kinshasa, Kinshasa, Democratic Republic of the Congo

Malaria remains a global problem and remains a major public health concern for the countries of Sub-Saharan Africa, particularly the Democratic Republic of Congo (DRC). It is one of the leading causes of morbidity including severe form occurs in individuals lacking premunition or those who have lost over several years without exposure , particularly children under 5 years and pregnant women . This form of malaria is based on high hospital mortality in a pediatric setting, requiring proper care, effective and consistent with national policy. This study aimed to describe the forms of severe malaria to determine the molecules used for the care and describe the evolution of children hospitalized for severe malaria. This descriptive study was conducted in the pediatric wards of General Reference Hospitals of Makala (GRHM), Kintambo (GRHK) and Universitary Clinics of Kinshasa (UCK) for the periods from 01 January 2011 to 13 July 2013 (2.5 years) by collecting information on archived records. Severe malaria cases in anemic and neurological forms were the most encountered respectively 58.59 % and 35.35 % in UCK; 62.2% and 30.8 % in GRHK; 65.5 % and 23 % in GRHM. Pulmonary and haemoglobinuric forms were also observed. Injectable quinine infusion was the most commonly used antimalarial molecule in 91.92 %; 89.7 % and 97.5% of cases respectively at UCK, GHRK and GHRM. The evolution after treatment showed a mortality of 33.3% (UCK), 23.4 % (GRHK) and 39 % (GRHM). But healing was observed in most cases at 67% (UCK), 67.1 % (GRHK) and 61 % (GRHM) without sequelae but 1.01% has sequelae In conclusion, the predominant shape was anemic form followed by neurological form, quinine antimalarial infusion was administered to support these and therapeutic evolution post cure was recovery in most cases, all times with considerable mortality. The ideal is an early treatment of malaria cases to avoid severe cases.

1470

ARE WE ACHIEVING SUFFICIENT POPULATION COVERAGE OF ARTEMISININ-COMBINATION THERAPY AMONG CHILDREN WITH MALARIA IN AFRICA? A SYSTEMATIC ANALYSIS OF DATA 2003-2012

Adam Bennett[1], Josh Yukich[2], Thomas P. Eisele[2]

[1]University of California San Francisco, San Francisco, CA, United States, [2]Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States

Artemisinin-based combination therapies (ACTs) are highly effective for curing uncomplicated malaria and preventing progression to severe disease. Funding for ACTs has dramatically increased and most countries in Africa have promoted ACT as first-line treatment since around 2005. Since this time, there has been a major rise in global ACT procurement. However, because of the challenges of reliably measuring the population coverage of ACTs among children with confirmed uncomplicated malaria, to date continent-wide changes in treatment coverage have not been quantified in a rigorous manner. Data from household surveys with parasite testing using antigen-detection rapid diagnostic tests (RDTs) are increasingly available, which provide a period prevalence estimate of infection that overlaps with two-week fever history. We combined data from 71 national household surveys (DHS, MIS, and MICS) to estimate the annual proportion of children with uncomplicated malaria (fever + parasite infection measured by RDT) receiving ACTs for all countries in sub-Saharan Africa 2003-2012. We used an individual-level logistic regression model including local PfPR, child age, household wealth, urban/rural, and insecticide-treated net (ITN) possession to predict RDT status for children in surveys without parasite testing. We used ACT distribution data combined with country-level covariates and temporally-correlated random effects in generalized linear regression models within a Bayesian framework to predict coverage to countries and years without data. Scale-up of treatment with ACTs among all children with uncomplicated malaria has been modest, reaching only 18% (95% Credible Interval 13%-22%) by 2011-2012, with highly variable coverage by country. The primary barriers to treatment with an ACT appear to be low treatment-seeking rates and inadequate access to health services, as coverage is much higher amongst children for whom care was sought. Additionally, children were more likely to receive an ACT in the public sector than in the private sector, but RDT+ children were only slightly more likely to have received an ACT than RDT- children, indicating a high degree of presumptive treatment. Improved access to health services and increased availability of ACTs in the private sector, coupled with increased demand for fever treatment, is critical for preventing severe disease and deaths among children with uncomplicated malaria.

1471

CHANGES IN THE AVAILABILITY AND AFFORDABILITY OF ACTS IN THE RURAL WEST AFRICAN PRIVATE RETAIL SECTOR: TWO AND A HALF YEARS POST AFFORDABLE MEDICINES FACILITY - MALARIA (AMFM)

Evelyn K. Ansah

Ghana Health Service, Accra, Ghana

The four main objectives of Affordable Medicines Facility - malaria (AMFm) were to: (i) to increase Artemisinin Combination Therapy (ACT) affordability; (ii) to increase ACT availability; (iii) to increase ACT use, including among vulnerable groups; and (iv) to "crowd out" oral artemisinin monotherapies. Ghana was among the nine countries which piloted the first phase of the strategy. The majority of adults and children with febrile illness, including the poorest are treated in the private retail sector. The study assessed changes in ACT availability in private retail shops 2 months before, 2 months after and 2.5 years after the arrival of the first co-paid ACTs in Ghana in August 2010. We also assessed prices of antimalarials (AM) in the shops 2.5 years after AMFm in a rural district in Ghana with an original fixed co-paid ACT price of GHC1.50. Supply, stock-out and cost issues were explored during the last survey in February 2013. Fifty-three chemical shops and 3 pharmacies out of 62 shops participated in the study. Overall, there were 398, 388 and 442 different brands of AMs in the shops during the 3 censuses. ACTs increased over the period, comprising 16.6%, 42.5% and 47.7% of AM in stock respectively. There appeared to be a slight reversal with regards to the market share of non artemesinin therapies from 34.2%, and 6.9% to 9.5% in the most recent census. Artemisinin monotherapies comprised of 9.5%, 4.6% and 3.4% AM available in the 3 time periods. Stocks of Herbal based AM preparations were relatively high forming 40-45% of all stock of AM. This did not change much over the period, constituting 39.7%, 45.9% and 39.4% of AMs respectively. For both children and adults, ACTs were the most sold AM type. Overall, 55.4% (31/56) of shops had experienced stock-outs of quality assured ACTs (QAACTs) in the preceding 2 months,

most of them (12/31; 38.7%) for a 1-2 week period. Sixteen of the 56 shops (28.6%) had no stock of QUAACTs. Buying and selling prices of QAACTs had increased by 40-100% and shopkeepers attributed this mainly to the scarcity of the commodity. In order to prevent reversal of the gains in malaria control over the last decade, consistent supply of QAACTs to the private retail sector must be assured.

1472

EFFECTIVENESS OF PROVIDER AND SCHOOL INTERVENTIONS ON THE TREATMENT PROVIDED TO FEBRILE PATIENTS ATTENDING PUBLIC PRIMARY HEALTH CENTRES AND MEDICINE RETAILERS IN SOUTHEASTERN NIGERIA

Obinna Onwujekwe[1], Lindsay Mangham-Jeffries[2], Bonnie Cundill[2], Neal Alexander[2], Julia Langham[2], Ogochukwu Ibe[1], Benjamin Uzochukwu[1], Virginia Wiseman[2]

[1]University of Nigeria, Enugu, Nigeria, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom

A formative survey for this study found that less than 1% of patients were tested for malaria, ACTs were received by only 22.4% of all patients and 37.9% of patients received SP. There was hence the need to improve appropriate treatment malaria by designing and implementing useful interventions. The interventions were evaluated using a three-arm cluster randomized trial in a real-life setting. The three arms were: the Control; Intervention arm 1; and Intervention arm 2. In the control arm there was only normal practice with supply of rapid diagnostic tests (RDTs) with basic instruction. In arm 1 there was provider intervention with supply of RDTs. In arm 2 there was provider intervention and school-based community intervention. The interventions were evaluated using a patient exit survey, log of malaria tests conducted, provider survey and a household survey. Within each stratum and arm, a point estimate of the proportion of patients treated according to guidelines was calculated. The implementation of the interventions differed in some stratum within the same arms. There was a general increase in testing compared to formative study, but the number of patients that were tested was still low across the three different arms despite the availability of RDTs in the facilities and there were no significant differences by arm. A large proportion of patients asked for a specific medicine and 96% of those who asked for a specific medicine got what they asked for. There was also no evidence of a difference between the intervention arms and control in the proportion of test positive patients receiving an ACT. It was found that 63% of those not tested asked for a medicine compared with 19% of those tested. The interventions did not make significant improvements in the intervention arms compared to the control arm. The reasons for this may include the real life setting of the project, the non-uniform implementation of the interventions in some arms and the relative differences in the gap between implementation and evaluation of the intervention in some clusters.

1473

HIGH FREQUENCY OF SUBMICROSCOPIC GAMETOCYTE CARRIAGE AFTER THE TREATMENT OF UNCOMPLICATED MALARIA WITH ACTS

Marielle K. Bouyou-Akotet[1], Denise P. Mawili-Mboumba[1], Rosalie Nikiema Ndong Ella[1], Eric Kendjo[1], Brigitte Charron[2], Valérie Lameyre[2], Maryvonne Kombila[1]

[1]Université des Sciences de la Santé, Libreville, Gabon, [2]Access to Medicine, Gentilly, France

Studying the parasite reservoir is a tool for monitoring the effectiveness of control strategies. The gametocyte carriage is more common in patients with asexual forms, and the occurrence of recrudescence or reinfection in patients could also be favored and contribute to the maintenance of a large reservoir of parasites. The aim of this study was to determine the prevalence of submicroscopic gametocytaemia in patients treated for uncomplicated malaria. Gametocytes carriage and density were estimated by Pfs25mRNA amplification using QT-NASBA in samples obtained at enrolment and during the follow-up (day 21 to day 42 post-treatment) in samples of children treated with either artesunate-amodiaquine (ASAQ) or artemether-lumefantrine (AL). Data were analyzed according to the study visit, the presence of asexual parasites, the type of treatment and the treatment response. Samples from 48 children were analyzed; 23 were treated with ASAQ and 25 with AL. They had 147 visits, all corresponding to treatment failure with either ASAQ or AL. None of the patients had a microscopic gametocytaemia. Overall, the frequency of SMG carriage was 51%, comparable at day 0 between the ASAQ (53%) and the AL (56%) patients (p=0.6). During the post-treatment visits, it was of 58% and 44% respectively in the ASAQ and in the AL groups respectively (p= 0.4). When pair samples of 23 children were analyzed, the gametocytaemia was positively correlated with the asexual form density the day of treatment failure (rho=0.4 in the ASAQ group and 0.5 in the AL). Logistic regression analysis showed that recrudescent infection (aOR: 12.9[1.1-14.9]) were independent risk factors for SMG carriage whereas no association was found with the type of treatment, age and number of episode. The frequency of SMG carriage is high after ACT treatment whatever the combination used. A strong association between the presence of gametocytes and a recurrent infection is also observed.

1474

LACK OF SIGNIFICANT PHARMACOKINETIC INTERACTIONS BETWEEN PIPERAQUINE AND NEVIRAPINE- OR EFAVIRENZ-CONTAINING ANTIRETROVIRAL REGIMENS IN *PLASMODIUM FALCIPARUM* NEGATIVE HIV-INFECTED MALAWIAN ADULTS STABILIZED ON HAART

Victor Mwapasa[1], Fraction Dzinjalamala[1], Jane Mallewa[1], Mavuto Mukaka[1], Saye Khoo[2], Robert Heyderman[3], David Lalloo[4]

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

In sub-Saharan Africa (SSA), most HIV-infected (HIV+) individuals on antiretroviral therapy (ART) are exposed to malaria. Currently, Dihydroartemisinin-piperaquine (DPQ) is being rolled out in SSA but no studies have examined the pharmacokinetics (PK) and safety of DPQ in HIV+ individuals taking ART containing Nevirapine (NVP) or Efavirenz (EFV). We conducted an open label clinical trial to compare the maximum concentration (Cmax) and area under concentration-time curve (AUC) of piperaquine (PQ) in antiretroviral naive HIV+ individuals and those taking NVP and EFV-based ART. In step 1 of the trial, malaria uninfected adults (n=6/ART group) received half the standard dose of DPQ (2 tablets of 40/320mg each for participants) at times 0, 24 and 48hrs. In Step 2, another group of malaria uninfected adults (n=15/ART group) received a standard dose of DPQ (4 tablets of 40/320mg each). Data-rich PK blood sampling were performed at the following times after dosing; 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, 36, 48, 60, 72, 96, 120, 144, 168, 336, 504 and 672 hrs. PQ levels were measured using HPLC-UV assays. We also assessed treatment emergent hematological and biochemistry abnormalities. The baseline demographic characteristics and CD4 cell counts were similar across the three groups. In step 1, compared with the ART-naïve group, there was a non-significant trend towards higher PQ AUC in the NVP-ART group and lower PQ AUC in the EFV-ART group. Similarly in Step 2, median PQ AUC was non-significantly lower in the EFV-ART group (15 µg/mL.hr, range: 2.6-25.6) than the ART-naïve group (22.6 µg/mL.hr: range: 11-37, p=0.052). The median PQ AUC in the NVP-ART group (29.9 µg/mL.hr, range: 14.2-80.9) was similar to the ART-naïve group (p>0.16). Cmax for PQ was similar across the three groups. In step 2, there were transient cases of grade 1 or 2 transaminitis in the ART-naïve arm and treatment-emergent grade 3 or 4 neutropenic episodes across the study arms. However, these abnormalities were not clinically significant nor persistent. Thus, there are limited PK interactions between DPQ and EFV or NVP-based ART.

1475

OVER-TREATMENT WITH ACTS AND FALSE ANTIMALARIAL DRUG HISTORY DETECTED THROUGH SERUM DRUG CONCENTRATION STUDIES

Akinwumi A. Akinyede[1], Peter Ojobor[2], Aderonke Ayanniyi[2], Olufunsho Awodele[2], Austin Adaje[2], Patrick Orumbie[3]

[1]University of Lagos & Babcock University, Lagos/Illishan-Remo, Nigeria, [2]University of Lagos, Lagos, Nigeria, [3]Lagos University Teaching Hospital, Lagos, Nigeria

Artemisinin Combination Therapies (ACTs) were recommended as first line therapy for patients with uncomplicated malaria fever in regions where chloroquine resistant strains of *Plasmodium falciparum* were found in the last 10 years. However, there is an emerging trend such that doctors' prescriptions contain more of chloroquine and other antimalarial agents either as monotherapy or in combination with ACTs forming triple therapies because physicians make presumptive diagnosis of resistance to ACTs. Antimalarial drug use histories of 18 adults with clinical diagnosis of uncomplicated malaria were taken in the staff clinic of the Lagos University Teaching Hospital, Nigeria. Blood samples were also tested for malaria parasitaemia and artemether/lumifantrine concentration on Day 0(pre-treatment) and Day 4 (day after completion of treatment).All patients declined the use of any antimalarial (including ACTs) during the 2 week period preceding the study and were malaria parasite negative on Days 0 and 4. Artemether was not detectable in the blood samples taken on Days 0 and 4. However, lumefantrine was detected in all blood samples taken on Days 0 and 4. The mean concentration of lumefantrine on Days 0 and 4 were 330.0µg/l±33.77 (SEM) and 349.7µg/l±18.39(SEM) respectively, these values were not significantly varied p> 0.05.This study exposed the wide spread use of artemether/lumefantrine among this group of patients before presentation at the clinic and underscores the need for confirmation of malaria parasitaemia before drug treatment. The patients' drug use history is also unreliable. Our findings may be a pointer to the fact that presumptive diagnosis of malaria resistant to ACTs should be halted even in malaria endemic regions such as Nigeria, these patients should have their blood tested for malaria parasites and blood drug concentration.

1476

HSP90, A POTENTIAL DRUG TARGET AGAINST *PLASMODIUM FALCIPARUM*

Claribel Murillo Solano, Shantanu Guha, Juan C. Pizarro

Tulane University, New Orleans, LA, United States

The prevalence of drug resistance represents a major threat against current efforts to control malaria. Therefore, the development of new drugs or the identification of new drug targets against *Plasmodium* is a priority to reduce the impact of malaria. Towards that end, we evaluated the *in vitro* effect against *P. falciparum* of available inhibitors against its heat shock protein 90 (HSP90); this chaperone is a key component of the parasite stress response and protein folding machinery. We determined the EC50 for several chaperone inhibitors *in vitro* with a fluorescent-based assay against two *P. falciparum* reference strains 3D7 and W2. The same method was used to determine their anti-Plasmodial effect in combination with current anti-malarial drugs. Moreover, the cytocidal activities of these compounds were evaluated in long term cultures following a bolus dosage exposure. The tested compounds were highly active against the malaria parasites with EC50 values between 10-7 to 10-5 M, and some of the compound-drug combinations displayed synergistic interactions inhibiting parasite growth. In parallel, we have cloned all the four genes coding for Hsp90 family members from *P. falciparum* and generated constructs to express the protein chaperones in bacteria. The recombinant proteins have been used to assay the inhibitors specificity in biochemical assays, aimed at determine their mechanism of action. The recombinant chaperones were screened against additional compound libraries to identify new compounds with potential anti-plasmodial activity. Our preliminary results, lead us to conclude that the *P. falciparum* Hsp90 chaperones is an appealing new drug target to combat malaria.

1477

EVALUATION OF ARTEMETHER PLUS LUMEFANTRINE TREATMENT FAILURES IN WEST AFRICA

Daouda Ndiaye[1], Davis Nwakanma[2], Baba Dieye[1], Yaya Ndiaye[1], Lansana Sangare[3], Fatou Joof[2], Clarissa Valim[4], Sarah K. Volkman[4], Dyann F. Wirth[4], Donald J. Krogstad[5]

[1]University Cheikh Anta Diop, Dakar, Senegal, [2]MRC Unit, Fajara, Gambia, [3]University of Bamako, Bamako, Mali, [4]Harvard School of Public Health, Boston, MA, United States, [5]Tulane University Health Sciences Center, New Orleans, LA, United States

Malaria caused by *Plasmodium falciparum* is a serious concern for public health and development in sub-Saharan Africa. To address these problems, a number of African countries have adopted artemisinin-based combination therapies (ACTs) as their first-line treatment for uncomplicated malaria. We have examined the effectiveness of Coartem for uncomplicated *Plasmodium falciparum* malaria in Gambissara in The Gambia, Dioro in Mali and Thiès in Senegal. These studies have enrolled participants 2-20 years of age with 2,000 to 199,999 asexual parasites per µl of blood who had no evidence of severe or complicated malaria. Primary endpoints include asexual parasite counts <25% of baseline by day 3, clearance of asexual parasites by day 7 and the absence of recurrent infection between days 8 and 42. Secondary endpoints included asexual parasite clearance times, *ex vivo* determinations of susceptibility and resistance to individual antimalarials; testing for drug resistance markers and for presumptively neutral markers (SNPs). From September 2011 to February 2013, we performed an open enrollment, multicenter study of the standard 3 day course of artemether + lumefantrine (AL) for uncomplicated *Plasmodium falciparum* malaria according to World Health Organization (WHO) guidelines. Follow-up visits were performed on days 1, 2, 3, 7, 14, 21, 28, 35 and 42 to evaluate clinical and parasitological results. These studies have now enrolled 328 subjects with uncomplicated *P. falciparum* malaria, who have been treated with arthemeter plus lumefantrine and followed for recurrent infection or other evidence of treatment failure. Of the 328 subjects enrolled, 19 have been lost to follow-up and 13 have developed recurrent infections between days 8 and 42. However, there have been no early treatment failures (on or before day 7). Twelve of the 13 subjects with recurrent infections had parasites at the time of recurrence with different genetic markers (using the SNP-based barcode). However, 1 subject had parasites with similar markers at the times of diagnosis and recurrence together with delayed parasite clearance on day 3. The isolates from this patient also had IC50s above the mean values for both artemether and lumefantrine. Apart from that subject, the results obtained thus far provide no evidence for artemisinin or Coartem resistance at the community level in The Gambia, Mali or Senegal.

1478

INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY WITH MEFLOQUINE IN HIV-INFECTED WOMEN RECEIVING COTRIMOXAZOLE PROPHYLAXIS: A MULTICENTER RANDOMIZED PLACEBO-CONTROLLED TRIAL

Raquel Gonzalez[1], Meghna Desai[2], Eusebio Macete[3], Peter Ouma[2], Mwaka Kakolwa[4], Salim Abdulla[4], John Aponte[1], Helder Bulo[3], Abdunoor M. Kabanywanyi[4], Abraham Katana[2], Sonia Maculuve[3], Alfredo Mayor[1], Arsenio Nhacolo[3], Kephas Otieno[2], Golbahar Pahlavan[1], María Rupérez[1], Esperança Sevene[3], Laurence Slutsker[5], Anifa Vala[3], John Williamsom[2], Clara Menéndez[1]

[1]Barcelona Centre for International Health Research (CRESIB), Barcelona, Spain, [2]Kenya Medical Research Institute/Centers for Disease Control and Prevention Research and Public Health Collaboration, Kisumu, Kenya, [3]Manhiça Health Research Center (CISM), Manhiça, Maputo, Mozambique, [4]Ifakara Health Institute, Dodoma, Dar Es Salaam, United Republic of Tanzania, [5]Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, United States

Intermittent preventive treatment in pregnancy (IPTp) with sulphadoxine-pyrimethamine (SP) is recommended for malaria prevention in HIV-negative pregnant women, but it is contraindicated in HIV-infected women due to potential interactions with cotrimoxazole prophylaxis (CTXp). We studied the safety and efficacy of mefloquine (MQ) in women receiving CTXp and long-lasting insecticide treated nets (LLITNs). A total of 1071 HIV-infected women from Kenya, Mozambique and Tanzania were randomized to receive either three doses of IPTp-MQ (15 mg/kg) or placebo given at least one month apart; all received CTXp and a LLITN. IPTp-MQ was associated with nearly halved maternal parasitemia (RR, 0.51 [95%CI, 0.29; 0.90]; p=0.021), and placental infection (RR, 0.53 [95%CI, 0.30; 0.93]; p=0.028), and reduced incidence of all-cause and non-obstetric hospital admissions (RR, 0.65 [0.41.; 1.03]; p=0.065; and RR, 0.59 [0.37; 0.95]; p=0.031; respectively). There were no differences in the prevalence of adverse pregnancy outcomes between groups. Drug tolerability was poorer in the MQ group compared to the control group (29.6% referred dizziness and 23.9% vomiting after the first IPTp-MQ administration). HIV viral load at delivery was higher in the MQ group compared to the control group (p=0.048). The rate of perinatal mother to child transmission (MTCT) of HIV was increased in women who received MQ (RR, 1.95 [95%CI 1.12;3.39]; p=0.018). An effective antimalarial added to CTXp and LLITNs in HIV-infected pregnant women can improve malaria prevention and maternal health through reduction in hospital admissions. The translation of this information into policy actions that reduce malaria in this particularly vulnerable group should be prioritized. However, MQ was not well tolerated, limiting its potential for IPTp and indicating the need to find alternatives. MQ was associated with an increased risk of MTCT of HIV, which warrants a better understanding of the pharmacological interactions between antimalarials and antiretroviral drugs.

1479

SIMULATIONS TO INVESTIGATE NEW INTERMITTENT PREVENTIVE THERAPY DOSING REGIMENS FOR DIHYDROARTEMISININ-PIPERAQUINE

Jesmin P. Lohy Das

Uppsala University, Uppsala, Sweden

A fixed-dose combination of dihydroartemisinin (DHA) and piperaquine (PQ) with monthly dosing has been suggested as a new promising alternative for Intermittent Preventive Therapy (IPT). Alternative dosing regimens for DHA-PQ was explored based on simulations with a previously developed in silico model describing the concentration-effect relationship for the malaria preventive effect. The model was developed in application to placebo controlled monthly versus bimonthly dosing regimen study of 1000 healthy male subjects in Northern Thailand. The simulations compared the clinically investigated monthly dosing regimen (120 mg DHA, 960 mg PQ dosing on three consecutive days repeated every month) to novel dosing regimens (120 mg DHA, 960 mg PQ once weekly). The usefulness of initial loading doses and robustness towards different levels of compliance was investigated for both weekly and monthly regimens. Among placebo recipient, the predicted yearly malaria incidence was 52%. In perfect compliance, the annual malaria incidence was less than 1% for weekly dosing compared to approximately 3% for the once monthly dosing regimen. Under the assumption of poor treatment compliance (60%), the weekly dosing of initial 3 day loading dose was predicted to contain the incidence below 3% compared to >15% for any monthly loading dose strategy in a year. Clinical trial simulations were applied to investigate the necessary sample size to confirm the predicted advantage with weekly compared to monthly dosing if a study was to be carried out under similar conditions as the original study. A samples size of 966 subjects (483+483) was needed to have 80% power to demonstrate a statistically significant benefit of weekly dosing over monthly dosing in a 9 months clinical trial. To have the same power to demonstrate non-inferiority (25% margin) a sample size of 684 subjects (342+342) was needed.

1480

PREGNANCY LOWERS THE EXPOSURE OF DIHYDROARTEMISININ: WHAT IS NEXT, INCREASE THE DOSE OR EXTEND THE TREATMENT?

Frank L. Kloprogge, Joel Tarning

Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand

Our work, comprising paired comparisons as well as literature comparisons between pregnant and non-pregnant women, has shown that the exposure of dihydroartemisinin is decreased during pregnancy after oral administration of dihydroartemisinin, artesunate and artemether. This is worrisome as these drugs are the first line treatment during the second and third trimester of pregnancy in many countries and decreased exposures can result in therapeutic failures and an accelerated development of resistance. The aim of this study was to evaluate the pharmacokinetics and pharmacodynamics of the artemisinin drugs in the treatment of uncomplicated malaria in pregnant women and investigate different optimised dose regimens. In-silico Monte-Carlo simulations, based on the pharmacokinetic exposures and pharmacodynamic parasite reduction, were used to evaluate the effect of a dose increase and treatment extension for oral administration of dihydroartemisinin, artesunate and artemether in pregnant women with uncomplicated *Plasmodium falciparum* malaria. Simulations indicated that it was possible to achieve similar dihydroartemisinin exposures in pregnant women compared to non-pregnant patients after an increased dose. However, this would also result in higher peak concentrations which may result in toxic side effects. An extended treatment could compensate for the lower dihydroartemisinin exposures during pregnancy without this increase in peak levels, but it may also result in lower adherence. This study suggests an optimised dose regimen for pregnant women with uncomplicated *P. falciparum* malaria. New pharmacokinetic studies evaluating the suggested dose optimisations are needed to enable an evidence-based dose optimisation.

1481

PKPD RELATIONSHIPS BETWEEN PLASMA PIPERAQUINE LEVELS AND CARDIAC QTC PROLONGATION IN MALARIA PATIENTS ADMINISTERED DIHYDROARTEMISININ-PIPERAQUINE IN CAMBODIA SUGGEST A CONVENTIONAL 3-DAY REGIMEN IS SAFER THAN A COMPRESSED 2-DAY REGIMEN

Pattaraporn Vanachayangkul[1], Jessica Manning[1], Chanthap Lon[1], Michele Spring[1], Mary So[2], Sok Sommethy[2], Sut-Thang Pann[3], Soklyda Chann[1], Mali Ittiverakul[1], Sabaithip Sriwichai[1], Nillawan Buathong[1], Worachet Kuntawunginn[1], Winita Ta-aksorn[1], Paktiya Teja-Isavadharm[1], Eugene Soh[4], Charlotte Lanteri[1], Jaranit Kaewkungwal[5], Douglas Tang[6], Mark Haigney[4], Louis Cantelena[4], David Saunders[1]

[1]Department of Immunology and Medicine, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]Royal Cambodian Armed Forces, Phnom Penh, Cambodia, [3]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia, [4]F. Edward Hebert School of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [5]Center for Excellence in Bioinformatics (BIOPHIC), Mahidol University, Bangkok, Thailand, [6]Fast Track Biologics, Bethesda, MD, United States

Dihydroartemisinin-piperaquine (DP), presently the firstline therapy for uncomplicated *Plasmodium falciparum* and *P. vivax* malaria in Cambodia, is widely used as a standard 3-day dosing regimen (360 mg dihydroartemisinin & 2880 mg piperaquine). However, piperaquine can prolong the QTc interval, resulting in cardiotoxicity that is undetected in countries like Cambodia lacking electrocardiograms. We conducted 3 clinical studies to explore the cardiotoxicity risk of administering DP for malaria treatment and prevention in Cambodia. Comparison of the 3-day versus a compressed 2-day DP regimen (total dose equivalent to 3-day DP), the latter used by the Cambodian military for malaria treatment, revealed both regimens had similar efficacy with mild correlation of plasma piperaquine-QTc prolongation. In a follow-on randomized, double-blind, placebo-controlled study evaluating 2-day DP as a monthly malaria prevention therapy, the trial was halted after 4 out of 69 volunteers met a pre-specified safety endpoint of >500 ms QTcF prolongation. Two-day DP had moderate correlation of plasma piperaquine with QTc prolongation (spearman rho = 0.6706, p-value < 0.0001), with strong correlation in the 4 halted volunteers (spearman ρ = 0.8990, p-value < 0.0001). In an ongoing 3-day DP treatment trial, we observe greater treatment failures and piperaquine IC_{50}s relative to our treatment study conducted 3 years prior, and note mild correlation of piperaquine-QTc prolongation (spearman Rho = 0.3954, p-value < 0.0001). A significant correlation between piperaquine-QTc prolongation was observed in a larger proportion of volunteers given 2-day DP (36 out of 47, 76.6%) relative to 3-day DP (13 out of 50, 26%). Mean plasma piperaquine C_{max} at 4 hours post-1st dose of 2-day DP (633.6 ng/ml) was significantly higher than for 3-day DP (127.3 ng/ml). Mean QTcF after 4 hr post-1st dose of 2-day DP (440.9 ms) was also significantly higher than 3-day DP (405.1 ms). Our findings suggest risk for cardiotoxicity can be mitigated by using 3-day DP, rather than a compressed regimen, with additional precautions of fasting and avoiding co-administration of other QT-prolonging medications.

1482

DEVELOPMENT OF PEDIATRIC FORMULATION FOR TREATMENT OF *PLASMODIUM FALCIPARUM* MALARIA: COARTEM® (ARTEMETHER-LUMEFANTRINE) DISPERSIBLE

Quique Bassat[1], Salim Abdulla[2], Bernhards Ogutu[3], Abdoulaye DJimde[4], Kirstin Stricker[5], Kamal Hamed[6], Heiner Grueninger[5]

[1]Barcelona Centre for International Health Research, Barcelona, Spain, [2]Ifakara Health Institute, Dar-es-Salaam, United Republic of Tanzania, [3]Walter Reed Project-Center for Clinical Research, Kenya Medical Research Institute, Nairobi, Kenya, [4]Malaria Research and Training Center, University of Bamako, Bamako, Mali, [5]Novartis Pharma AG, Basel, Switzerland, [6]Novartis Pharmaceuticals Corporation, East Hanover, NJ, United States

Pediatric artemisinin-based combination therapy formulations have the potential to improve effectiveness and accuracy of dosing in young children. Coartem® (artemeter-lumefantrine; AL) Dispersible was developed in partnership with the Medicines for Malaria Venture for the treatment of uncomplicated *Plasmodium falciparum* malaria and is the first pediatric antimalarial to receive Swissmedic approval and meet WHO specifications for use in infants and children ≥5 kg. Search using PubMed, Ovid and clinical trial registry databases for AL dispersible in children revealed 6 original and 5 review articles involving 674 infants/children. In a palatability study, sweet tasting cherry was the preferred flavor by children for AL dispersible. Pharmacokinetic profile of AL dispersible was comparable to AL crushed tablets. Efficacy and safety of dispersible formulation versus crushed tablet was evaluated in a large, randomized, multicenter study in 5 sub-Saharan African countries. Efficacy and acceptability of AL dispersible were also compared to dihydroartemisinin-piperaquine (DP) pediatric in an open-label, randomized study in Kenya. A total of 674 children were randomized in both studies to receive AL dispersible with mean age 38.5 months, body temperature 38.2°C and parasite density 38,202-53,921/µl. 28- and 42-day PCR-corrected cure rates were 97.8% and 96.4%; similar to AL crushed tablets and DP in respective studies. Acceptability of AL dispersible was significantly better than DP pediatric (ease of use: p=0.007; taste of medicine: p=0.001). 28-day PCR-corrected cure rate was not related to food intake; however, consumption of milk/low fat meal increased lumefantrine bioavailability compared to no food. Efficacy of AL dispersible was comparable in children with different body weights. Median parasite and fever clearance times were 34.3 and 7.9 hours (n=447). Safety profile of AL dispersible was comparable to crushed tablets. AL dispersible was specifically tailored for the pediatric population and offers a convenient formulation with efficacy and safety similar to that of standard crushed AL tablets.

1483

EFFICACY, SAFETY AND POPULATION PHARMACOKINETICS OF THE ARTESUNATE MEFLOQUINE (ASMQ) FIXED DOSE COMBINATION VERSUS ARTEMETHER LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED FALCIPARUM MALARIA IN AFRICAN CHILDREN

Sodiomon Sirima[1], Bernhards Ogutu[2], John Lusingu[3], Ali Mtoro[4], Zakayo Mrango[5], Alphonse Ouedraogo[1], Jean Baptiste Yaro[1], Kevin Omondi Onyango[6], Samwel Gesase[3], James Samwel Ngocho[5], Joelle Vanraes[7], Nathalie Strub[7], Gwenaelle Carn[8]

[1]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso, [2]The Kenya Medical Research Institute, Kisumu, Kenya, [3]National Institute for Medical Research, Korogwe, United Republic of Tanzania, [4]Ifakara Health Institute, Bagamayo, United Republic of Tanzania, [5]National Institute for Medical Research, Kilosa, United Republic of Tanzania, [6]The Centre for Clinical Research - Kenya Medical Research Institute, Nairobi, Kenya, [7]Drugs for Neglected Diseases initiative, Geneva, Switzerland, [8]Centre National de Recherche et de Formation sur le Paludisme, Geneva, Switzerland

Artemisinin-based combination therapies (ACTs) are recommended by WHO to treat uncomplicated *Plasmodium falciparum* malaria. Artesunate

(AS) and Mefloquine (MQ), in loose or fixed-dose combination (ASMQ), was the first ACT used extensively in Asia and Latin America but there is very limited data in Africa. Our objective was to evaluate the efficacy and safety of ASMQ in comparison with the standard of care Artemether-Lumefantrine (AL), and to study the population-pharmacokinetics (PK) in African children.The clinical trial was conducted in children aged from 6 months to 5 years in Burkina Faso, Kenya and Tanzania. Febrile children with *P. falciparum* density between 2,000 and 200,000 asexual parasites/µl were randomized to receive (a) ASMQ for 3 days [6 to 11 months old: one 25mg/55 mg tablet once daily (OD); 12 to 59 months old: two tablets OD], or (b) AL for 3 days [children 5-15 Kg: one 20mg/120mg tablet BID; 15-25 Kg: two tablets BID]. All children were followed for 60 days after treatment period. The primary efficacy outcome is the cure rate based on the PCR-adjusted results by Day 63. Cure rates at 28 and 42 days are also evaluated. Patients with parasitaemia during the follow-up period were switched to the other treatment arm and followed for a further 60 days or until second recurrence. Safety was assessed during the first follow-up period (up to Day 63) and during the second one if recurrence occurred. 945 patients were randomised by June 2013. Under blinded conditions the overall safety profile did not reveal any unexpected signals. The data base lock is expected mid 2014. The population PK results of ASMQ were presented in 2013, and showed a large inter-patient variability in children; clearance and volume of distribution of MQ in children is lower than in adult patients but the terminal elimination half-life and mean absorption time are of similar magnitude.

1484

A PHASE III, RANDOMIZED, OPEN LABELLED, ACTIVE CONTROLLED, MULTI CENTER, SUPERIORITY TRIAL OF ARTIMIST™ VERSUS INTRAVENOUS QUININE IN CHILDREN WITH SEVERE OR COMPLICATED FALCIPARUM MALARIA, OR UNCOMPLICATED FALCIPARUM MALARIA WITH GASTROINTESTINAL COMPLICATIONS

Stephen Rulisa[1], Patrick Ansah[2], Sirima, Ouagadougou, Burkina Faso B. Sodiomon[3]

[1]Kigali University Teaching Hospital, Kigali, Rwanda, [2]Navrongo, Navrongo, Ghana, [3]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso

Malaria is a serious, infectious disease. About half the world's population (3.3bn) live in areas that have some risk of malaria transmission, 36.4% (1.2bn) of which are living in regions considered at high risk The Phase III trial was carried out in malaria endemic areas of Rwanda, Burkina Faso and Ghana over a 22-month period from November 2010 to September 2012 151 subjects were randomised and enrolled. A total of 151 subjects were analysed in the Safety Analysis Population, 141 subjects in the Modified Intention to Treat (MITT) Population, and 137 subjects in the Per Protocol (PP) Population The study's primary objective was to demonstrate that sub lingual (under the tongue) ArTiMist™ was superior to IV quinine in reduction of the parasite counts by >90% within 24 hours in children with severe or complicated falciparum malaria, or uncomplicated falciparum malaria with gastrointestinal complications. The primary objective for this study showed that ArTiMist™ demonstrated superiority over iv quinine in both efficacy populations. For the MITT population 66 of the 70 subjects (94.3%) treated with ArTiMist™ and 28 of the 71 subjects (39.4%) treated with quinine had parasitological success. The absolute difference (95% CI) between treatments, without correcting for the factor site, was 54.85 (42.25 - 67.45) % which was statistically significant ($p < 0.0001$). For the PP population 65 of the 68 subjects (95.6%) treated with ArTiMist™ and 28 of the 69 subjects (40.6%) treated with quinine had parasitological success. The absolute difference (95% CI) between treatments, without correcting for the factor site, was 55.01 (42.44 - 67.58) % which was statistically significant ($p < 0.0001$). Following sublingual administration of ArTiMist™, absorption is rapid with mean Cmax following the first dose reaching 333.2 ng/mL and 83.3 ng/mL in 1.0 h and 1.5 h for artemether and dihydroartemisinin (DHA), respectively. In conclusion, sub-lingual ArTiMist™ was superior to IV quinine, demonstrating significantly faster parasite killing and fewer early treatment failures. PK analysis demonstrated that ArTiMist™ was rapidly absorbed in children with severe or complicated falciparum malaria, or children with uncomplicated malaria with gastrointestinal complications

1485

INHALED NITRIC OXIDE FOR THE ADJUNCTIVE TREATMENT OF SEVERE MALARIA: A RANDOMIZED CONTROLLED TRIAL

Michael Hawkes[1], Andrea Conroy[2], Laura Hermann[2], Chloe McDonald[2], Hani Kim[2], Sarah Higgins[2], Sophie Namasopo[3], Chandy John[4], Christopher Miller[5], W. Conrad Liles[6], Robert Opoka[7], Kevin Kain[2]

[1]University of Alberta, Edmonton, AB, Canada, [2]University of Toronto, Toronto, ON, Canada, [3]Jinja Regional Referral Hospital, Jinja, Uganda, [4]University of Minnesota, Minneapolis, MN, United States, [5]University of British Columbia, Vancouver, BC, Canada, [6]University of Washington, Seattle, WA, United States, [7]Makerere University, Kampala, Uganda

Severe malaria remains a major cause of childhood mortality globally. Exogenous inhaled nitric oxide (iNO) reduces endothelial activation, protects the blood-brain barrier, and improves survival in pre-clinical studies of experimental cerebral malaria. We conducted a randomized, blinded, parallel-arm, controlled trial of iNO at 80 ppm by non-rebreather mask vs room air placebo as adjunctive treatment in children (age 1 to 10 years) with severe malaria. Blinding of trial clinicians, nurses, parents, children, laboratory technicians and statistician was achieved by using room air placebo, also administered by mask by a dedicated and unblinded team that monitored dose-dependent adverse effects (methemoglobinemia) but did not participate in clinical care. The primary outcome was the rate of improvement in angiopoietin-2 levels (a biomarker of malaria severity and convalescence). 180 children were enrolled; 88 were assigned to nitric oxide and 92 to placebo (all received IV artesunate). The median [IQR] rate of change of Ang-2 over the first 72 hours of hospitalization was similar between groups: -2.2 [-3.1 to -1.2] ng/mL/day in the iNO group vs -1.9 [-3.7 to -0.56] ng/mL/day in the placebo group; p=0.68). The mortality at 48 hours was similar between groups (6/87 [6.9%] in the iNO group vs 8/92 [8.7%] in the placebo group; OR 0.78, 95% CI 0.26-2.3; p=0.65). Methemoglobinemia (>10%) was higher in the iNO group (5/88 [5.7%] vs 0/92 [0%]; p=0.026). Incidence of neurologic sequelae (<14 days), acute kidney injury, hypoglycemia, anemia and hemoglobinuria were similar between groups (p>0.05 for all comparisons). Clinical recovery times (time to eat, sit, localize pain, fever resolution, recovery of consciousness, and hospital discharge) were similar between group (p>0.05 for all comparisons). Parasites cleared quickly in both groups, with no difference in parasite clearance kinetics (p>0.05). No patient in either group had recrudescence of patent parasitemia at day 14 of follow-up. Inhaled nitric oxide at 80 ppm administered by non-rebreather mask was safe but did not accelerate endothelial stabilization, as reflected by circulating levels of Ang-2, in children with severe malaria. Alternative methods of delivering NO to the endothelium (e.g., higher dose, donor molecules, routes of administration) may be necessary to achieve a more potent biological effect and an impact on clinical outcomes.

1486

ALLOMETRIC SCALING OF PYRONARIDINE PHARMACOKINETIC PARAMETERS IN PEDIATRIC MALARIA PATIENTS

Amal Ayyoub[1], Janthima Methaneethorn[1], Stephan Duparc[2], Isabelle Borghini-Fuhrer[2], Jang-Sik Shin[3], Lawrence Fleckenstein[1]

[1]University of Iowa, College of Pharmacy, Iowa City, IA, United States, [2]Medicines for Malaria Venture, Geneva, Switzerland, [3]Shin Poong Pharmaceuticals, Seoul, Republic of Korea

Pyramax® is a pyronaridine/artesunate combination for the treatment of uncomplicated malaria in adult and pediatric patients. A granule formulation of this combination is being developed for treatment of

uncomplicated *Plasmodium falciparum* and *P. vivax* malaria in pediatric patients. The population pharmacokinetics of pyronaridine (PYR) were evaluated in pediatric malaria-infected patients participating in six Pyramax® clinical trials. A total of 1085 blood PYR concentrations were available from 349 malaria patients younger than 16 years of age with mild to moderate uncomplicated malaria. Blood PYR concentrations were measured using a validated LC-MS method. Non-linear mixed effects modeling was used to obtain the pharmacokinetic and variability parameter estimates. PYR concentrations were well described by a two-compartment model with first order absorption and elimination. Allometric scaling was implemented to address the effect of body weight on clearance and volume parameters. The final parameter estimates of PYR apparent clearance (CL/F), central volume of distribution (V2/F), peripheral volume of distribution (V3/F), inter-compartmental clearance (Q/F) and absorption rate constant (Ka) were 377 L/day, 2230 L, 3230 L, 804 L/day and 17.9 day-1, respectively. The corresponding percent coefficient of variation of inter-individual variability for CL/F, V2/F, V3/F and Ka were 40.7%, 99.6%, 50.6% and 65.8%, respectively. Covariate model building conducted using forward addition ($p<0.05$) followed by backward elimination ($P<0.001$) yielded two significant covariate-parameter relationships: age on V2/F and formulation on Ka. Evaluation of bootstrapping, visual predictive check, and condition number indicated that the final model displayed satisfactory robustness, predictive power, and stability. Simulations of PYR concentration-time profiles generated from the final model show similar exposures across pediatric weight ranges, supporting the proposed labeling for weight-based dosing of Pyramax® granules.

1487

EFFECTS OF BREATHING NITRIC OXIDE AS AN ADJUNCTIVE TREATMENT FOR CHILDREN WITH CEREBRAL MALARIA

Juliet Mwanga-Amumpaire*[1], Ryan W. Carroll[2], Elisabeth Baudin[3], Elisabeth Kemigisha[1], Dan Nyehangane[1], Pierre De Beaudrap[4], Jean-François Etard[3], David Bangsberg[2], Kenneth Bloch[2], Yap Boum II[1], Warren Zapol[2]

[1]Epicentre Research Base of Mbarara/Mbarara University of Science and Technology, Mbarara, Uganda, [2]Massachusetts General Hospital/Harvard Medical School, Boston, MA, United States, [3]Epicentre, Paris, France, [4]Epicentre Research Base of Mbarara, Mbarara, Uganda

Children with cerebral malaria (CM) have reduced plasma and urine levels of NO biometabolites. Two studies have reported breathing NO reduces mortality, inflammation, and CNS pathology in *Plasmodium berghei*-infected mice. Therefore, from Sept 2011 to February 2014, we completed a phase-II open-label clinical trial assessing the efficacy and safety of inhaled NO (INO) as an adjunctive treatment for cerebral malaria in pediatric patients. A total of 92 children, aged 3 months – 9 years, with CM were enrolled in the study at the Mbarara Regional Referral Hospital in Uganda. Patients were randomly assigned to receive either inhaled NO (INO), or nitrogen (N_2) via nasal cannula (INOPulse, TM, Ikaria, USA) for at least 24 hours. All patients received IV artesunate and were monitored continuously for changes in metHb% levels. The primary endpoint was the change in plasma Angiopoietin-1 (Ang-1) over 48 hours. Plasma Ang-1 levels increased over 48 hours in both treatment groups, but there was no difference between the groups. There was a decrease in plasma angiopoietin-2 and plasma cytokine levels (TNF-α, IFN-γ, IL-1β, IL-6, IL-10, and MCP-1) over 48 hours in both study arms, but no significant difference between the treatment groups. Total mortality was 12.0%. Seven (15.2%) patients died in the N_2 group, and 4 (8.7%) patients died in the INO group. Five patients in the N_2 group and 6 in the INO group had developed neurological sequelae by the time of discharge. For patients who received INO, the average hourly dose of INO delivered over the initial 48 hours was 1.13 ± 0.30 mg/kg/hr (N=39, mean $\pm$ SD). There was no difference in the baseline metHb% levels among patients in both study arms. There was an increase in metHb% in patients treated with INO, up to 4.1 ± 2.3% (N=33, mean $\pm$ SD) at 12 hours, which remained at safe elevated levels up to 72 hours. MetHb levels were unchanged throughout the treatment period in the N_2 group. This pilot trial of INO as an adjuvant therapy for CM demonstrates the safety and feasibility of delivering INO in a low-resource setting but there was no statistically significant evidence for efficacy.

1488

EXPERIMENTAL VIVAX TRANSMISSION TO *ANOPHELES* (EVITA), A CLINICAL TRIAL TO ASSESS MOSQUITO TRANSMISSIBILITY IN PARTICIPANTS INOCULATED WITH BLOOD STAGE *PLASMODIUM VIVAX*

Paul Griffin[1], Suzanne Elliott[2], Silvana Sekuloski[1], Cielo Pasay[1], Maggy Sikulu[1], Leon Hugo[1], Tom Churcher[3], Jetsumon Prachumsri[4], Ivinson Karen[5], Chris Ockenhouse[5], James McCarthy[1]

[1]Queensland Institute of Medical Research, Brisbane, Australia, [2]Qpharm Pty Ltd, Brisbane, Australia, [3]Imperial College London, London, United Kingdom, [4]Mahidol University, Bangkok, Thailand, [5]PATH, Malaria Vaccine Initiative, Washington, DC, United States

Interventions to interrupt transmission of malaria from humans to mosquitoes, including vaccines, represent appealing approaches to assist its elimination. A limitation has been the lack of a methodology to reliably test the efficacy of such interventions before proceeding to clinical efficacy trials in the field. Building on our work demonstrating the feasibility of induced blood stage *Plasmodium vivax* infection, we have undertaken a study to evaluate transmission to *Anopheles stephensi* mosquitoes. Study endpoints included the presence of the gametocyte-specific transcript *pvs25* in the blood of volunteers by qRT-PCR, and mosquito infection by midgut dissection for oocyst visualisation. The study design entailed 3 cohorts of 2 volunteers, each inoculated on Day 0 with approximately 100 viable *P. vivax*-infected human erythrocytes administered intravenously. On the three to four days up to the anticipated commencement of treatment (approximately day 11, 12, 13 and 14), transmission studies were undertaken by membrane feeding assays and direct feeds on volunteers with 30 mosquitoes per session. At the time of abstract submission, 2 of the 3 cohorts have been completed. No significant adverse events were observed. All subjects experienced mild to moderate symptoms of malaria consistent with previously published data. Direct mosquito feeding was well tolerated with all volunteers reporting mild to moderate local reactions and pruritis, easily controlled with symptomatic treatment. Elevated liver function tests were observed in 3 of the 4 volunteers in the form of asymptomatic elevations in both ALT and AST. However, this completely resolved in all subjects. Gametocytaemia detected by a positive pvs25 RT-PCR was observed in all subjects. Mosquito infection was detected by midgut dissection following both direct and indirect feeding assays. The demonstration of the feasibility of this system to test transmission-blocking interventions represents a promising development for future efficacy studies.

1489

SAFETY AND REPRODUCIBILITY OF AN INDUCED BLOOD STAGE MALARIA CHALLENGE FOR EXPEDITED TESTING OF ANTIMALARIAL TREATMENT AND VACCINES

Paul Griffin[1], Silvana Sekuloski[1], Suzanne Elliott[2], Cliodna NiBuachalla[2], Casey Rowe[2], David Khoury[3], Deborah Cromer[3], Miles Davenport[3], James McCarthy[1]

[1]Queensland Institute of Medical Research, Brisbane, Australia, [2]Qpharm Pty Ltd, Brisbane, Australia, [3]The University of New South Wales, Sydney, Australia

Despite recent progress in malaria control there is concern that momentum is slowing and new challenges are emerging, including the development of drug resistance. Thus, new interventions are required. Controlled Human Malaria Infection (CHMI) studies are assuming an increasing place in evaluation of interventions as they can lead to accrual of pivotal efficacy data in a faster and more cost-effective fashion than Phase IIa studies in endemic settings. An alternative to infection via sporozoite, either by

mosquito bite or injection of cryopreserved sporozoites, Induced Blood Stage Malaria (IBSM) infection represents a convenient approach where pre-erythrocytic stages are not being studied. Here we report on the safety and reproducibility data from 121 subjects in 12 trials from our centre, the largest report of IBSM. The majority of subjects (86%) experienced at least some symptoms of malaria infection. In total 755 adverse events were recorded however the majority (75%) were mild. No SAE's were attributed to malaria with 4 SAE's unrelated to trial protocol and 3 SAE's attributed to the investigational product. Despite the positive serostatus of the donor for CMV, and the inclusion of seronegative subjects, no CMV seroconversions were detected nor were any additional coinfections observed on extensive serological testing. Analysis of parasitaemia by sensitive qPCR demonstrates that the method is reliable and reproducible. Further analysis of the reproducibility and variability of parasite growth rates is currently underway and will be presented. These data illustrate the safety and reproducibility of an induced blood stage malaria model thus providing a valuable tool for assessing candidate drugs and vaccines for control of malaria.

1490

A PHASE IIA CLINICAL TRIAL TO CHARACTERIZE THE PHARMACOKINETIC-PHARMACODYNAMIC RELATIONSHIP OF PIPERAQUINE USING THE INDUCED BLOOD STAGE INFECTION MODEL

James S. McCarthy[1], Silvana Sekuloski[1], Paul Griffin[2], Suzanne Elliott[2], Louise Marquart[1], Joerg Moehrle[3], Mark Baker[3]

[1]QIMR Berghofer Medical Research Institute, Herston, Australia, [2]QPharm Pty Ltd, Herston, Australia, [3]Medicines for Malaria, Geneva, Switzerland

Piperaquine (PQP) is a 4-aminoquinoline antimalarial structurally related to chloroquine. It was widely used for malaria control in China in the 1970's and 1980's, and more recently has undergone renewed development as component of an ACT co-formulation with dihydroartemisinin (DHA-PQP). Although knowledge of the pharmacokinetic-pharmacodynamic (PK-PD) relationship of antimalarials is essential for dose selection, there is a paucity of such data for PQP. We therefore undertook an experimental dose de-escalation clinical trial of this drug in the induced blood stage infection malaria (IBSM) system, with simultaneous measurement of drug levels and parasitemia, the latter by qPCR. The trial was designed to include 3 single dose cohorts, each of 8 volunteers. The pharmacokinetic profile of PQP following single doses of 960 and 640 mg was linear with CL/f = 89 L/hr (95%CI: 75-101 L/hr), with measurable plasma levels (>1 ng/mL) out to 672 hrs following administration of 640 mg. Recrudescent parasitemia occurred after ≥144 hours in 4 of the 7 volunteers who received 640 mg PQP; each received rescue treatment with artemether/lumefantrine. The rich dataset accrued facilitated the fitting of a PK/PD model to the PK and parasitemia data. The concentration response relationship identified by analysis of data from the 960 and 640 mg cohorts was characterized by a PRR of 3.3 (95%CI: 3.0-3.6; t1/2: 4.4 hr), an IC50 of 9.2 ng/mL (95%CI: 7.1-11.9), an MPC of 14.3 ng/mL (95%CI: 11.0-18.5 ng/mL), and an MIC of 8.1 ng/mL (95%CI: 6.3-10.5 ng/mL). The model accurately predicted the parasitemia response observed in the 480 mg PQP cohort. The IBSM system demonstrated that the PK/PD relationship of an antimalarial can be determined from data obtained from just two cohorts of 8 volunteers. The compiled PK/PD model can then be linked with safety data to forecast optimal dosing, either as a single agent or in combination, whilst accounting for effects of age, DDI and parasitemia.

1491

DELAYED ANEMIA ASSESSMENT IN PATIENTS TREATED WITH ORAL ARTEMISININ DERIVATIVES FOR UNCOMPLICATED MALARIA: A POOLED ANALYSIS OF CLINICAL TRIALS DATA FROM MALI

Issaka Sagara[1], Renaud Piarroux[2], Abdoulaye Djimde[1], Roch Giorgi[2], Kassoum Kayentao[1], Ogobara K. Doumbo[1], Jean Gaudart[2]

[1]University of Bamako, Bamako, Mali, [2]Aix-Marseille University, Marseille, France

In sub-Saharan Africa, Artemisinin-based combination therapies (ACT) and injectable artesunate are the first line treatments for uncomplicated and severe *Plasmodium falciparum* malaria, respectively. However, recent studies suggest that delayed anemia is associated with these treatments in non-immune travelers. We aimed to assess the risk factors associated with delayed anemia after falciparum malaria treatment with artemisinin-containing drugs in malaria endemic populations. Pooled, individual malaria patient data were extracted from 13 clinical trials performed from 2002 to 2011 in various settings of Mali. Treatment regimens were Artemether-Lumefantrine, Artesunate plus Amodiaquine, Artesunate plus Sulfadoxine-Pyrimethamine, Artesunate plus Sulfamethoxypyrazine-pyrimethamine, Artesunate plus Mefloquine, Artesunate-Pyronaridine, Artesunate monotherapy, Chloroquine, Sulfadoxine-pyrimethamine, Amodiaquine and Sulfadoxine-pyrimethamine plus Amodiaquine. Univariate and multivariate analyses were performed using the generalized linear and latent mixed model procedures to assess risk factors associated with hemoglobin concentration evolution and anemia during the treatment follow-up. A total of 5990 participants were recruited and followed from Day 0 to Day 28. The participants' median age was 5 years, ranging from 3 months to 70 years. There was a decrease in hemoglobin level on day 7 in all treatments arms, but the magnitude varied across treatments. There was a significant risk of hemoglobin level decrease on day 7 in the artemisinin-based therapies compared to the non-artemisinin treatments. The risk of hemoglobin concentration drop was associated with age group < 5 years old (0.61 g/dL 95% CI [0.71 to 0.51], p<0.001), baseline high parasite density (0.43 g/dL 95% CI [0.51 to 0.35], p<0.001) and treatment failure (0.40 g/dL 95% CI [0.59 to 0.20], p=0.018), while high hemoglobin level at baseline was a protective factor [0.53 to 0.59] p<0.001). No association was found between artemisinin-based therapies and severe delayed anemia. Oral artemisinin derivative treatments for uncomplicated *P. falciparum* malaria are associated with a transient and clinically moderate hemoglobin decrease by day 7 but not associated with a delayed severe anemia.

1492

ARTEMISININ PARTNER'S DRUGS DAY 7 CONCENTRATION PROFILE AND ITS EFFECT ON RECURRENT EPISODES OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

Mamadou M. Tekete[1], Abdoulaye Djimde[2], Jürgen Burhenne[3], Issaka Sagara[2], Bakary Fofana[2], Omar Bila Traore[2], Souleymane Dama[2], Sekou Toure[2], Nianwalou Dara[2], Bouran Sidibe[2], Ogobara K. Doumbo[2], Walter Haefeli[3], Steffen Borrmann[1]

[1]Institute for Tropical Medicine, University of Tübingen, Tübingen, Germany, [2]University of Sciences, Techniques and Technologies of Bamako, Mali, Bamako, Mali, [3]Department of Clinical Pharmacology and Pharmacoepidemiology, University of Heidelberg, Heidelberg, Germany

Bougoula, Kolle, Sotuba are three sites in Mali participating in a large Phase III/VI trial of four ACTs (Artesunate-amodiaquine, artemether-lumefantrine, Dihydroartemisinin-piperaquine and Artesunate-pyronaridine). The role of the long half-life partner drugs is to mop up any remaining blood stage parasite biomass after treatment. Whether the long half-life may lead to accumulation when ACTs are used frequently in high transmission settings is currently not known but potentially important for drug safety and for the duration of post-treatment prophylactic. Patients with uncomplicated *Plasmodium falciparum* malaria, aged ≥6 months,

after inclusion in one of the treatment arms are followed up for two years, during which patients will receive the same treatment for any subsequent episode of malaria occurring at least 28 days after the start of the previous treatment. To date we have plasma concentrations in day 7 samples from 317 treatment episodes for desethyl-amodiaquine and 564 episodes for lumefantrine. Our first results show an increase of desethyl-amodiaquine concentrations from the first episode to consecutive episodes of malaria treatment with a median (quartile range) concentration of 70.6 ng/ml (58.8 to 89.1 ng/ml) (n=102) for the first, 90.8 ng/ml (69.0 to 111.0 ng/ml) (n=80) for the second; 80.2 ng/ml (61.8 to 100.3 ng/ml) (n=32) for the third and 97.7 ng/ml (82.2 to 1293.0 ng/ml) (n=23) for the fourth episode P<0.0001. For lumefantrine, there was no difference between the first and second episode 632.1 ng (405.7 to 948.6 ng/ml; n=343) and 697.15ng/ml (491.69 to 967.93ng/ml; n=135). There was, however, an increase between first and third episodes (789.3ng/ml; 574.3 to 1362.9 ng/ml; n=52; P = 0.002). All patients with day 7 concentration of lumefantrine below 100 ng/ml had recurrence of infections before day 42 of follow-up. These preliminary data show substantial accumulation of desethyl-amodiaquine in the study population exposed to frequent re-treatments in an area of intense seasonal malaria transmission. A larger dataset will be available at the meeting, including detailed analyses of laboratory parameters of safety and a survival analysis of time to recurrence corrected by transmission season, parasite genotypes (to distinguish recrudescent primary infections from new infections) and drug plasma concentrations.

1493

MEASURING THE EFFICACY OF FOUR ACT REGIMENS IN MALI USING QPCR-BASED ESTIMATES OF *PLASMODIUM FALCIPARUM* CLEARANCE TIME

Khalid B. Beshir[1], Nouhoum Diallo[2], Bakari Fofana[2], Aliou Traore[2], Aly Kodio[2], Amadou Togo[2], Nianwalou Dara[2], Moctar Coulibaly[2], Francois Dao[2], Issaka Sagara[2], Colin Sutherland[1], Abdoulaye Djimde[2]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Malaria Research and Training Centre, Department of Epidemiology of Parasitic Diseases, Faculty of Pharmacy, Bamako, Mali

The efficacy of artemisinin combination therapy remains high in sub-Saharan Africa, but the prolongation of parasite clearance times of artemisinin-treated *Plasmodium falciparum* infections in Cambodia and neighboring countries is a warning that careful monitoring of efficacy is required Worldwide. As an alternative to laborious frequently-spaced blood sampling and evaluation by standard light microscopy, we have developed a qPCR-based parasite clearance assay that utilizes daily finger-prick dried blood spots for the first 72 hours of treatment, and which underwent a successful proof-of-principle trial in western Kenya. We have now applied this approach to evaluate parasite clearance in over 200 falciparum malaria patients treated with artemisinin-combination therapy in two sites in Mali: Bougoula and Kolle. All patients were participants in efficacy evaluations by the WANECAM project, and were randomised to receive either artemether-lumefantrine, dihydroartemisinin-piperaquine, amodiaquine-artesunate or artesunate-pyronaridine for all malaria episodes during two years of follow-up. Parasite clearance estimates for 209 first malaria episodes and 186 second episodes across the two sites will be presented, and estimates of the parasite reduction ratio at 48 hours derived for each episode. These data will be analysed with reference to site, regimen received, patient age and, for second episodes, time elapsed since first episode.

1494

STUDY ON EFFICACY OF ARTESUNATE-MEFLOQUINE COMBINATION THERAPY FOR TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN THAILAND AS PART OF A DEPARTMENT OF DEFENSE MULTI-CENTER TRIAL

Krisada Jongsakul[1], Delia Bethell[2], Michele Spring[1], Ilin Chuang[1], Sha Law[3], Sabaithip Sriwichai[1], Worachet Kuntawunginn[1], Saowaluk Wongarunkochakorn[1], Alaina Thomas[4], Donna Tosh[5], David Saunders[1], Mark Fukuda[6], James Cummings[4]

[1]Armed Forces Research Institute of Medical and Sciences, Bangkok, Thailand, [2]Department of Paediatrics, Oxford Children's Hospital, Oxford, United Kingdom, [3]Kwai River Christian Hospital, Kanchanaburi, Thailand, [4]Armed Forces Health Surveillance Center, Silver Spring, MD, United States, [5]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [6]United States Agency for International Development, Bangkok, Thailand

Artemisinin-resistant *Plasmodium falciparum* threatens effectiveness of all artemisinin-based combination therapies. A multi-center artesunate-mefloquine (A+M) efficacy trial is ongoing in three DoD laboratories in Peru, Kenya, and Thailand, to compare parasite clearance rates for 72 hour after artesunate initiation and to conduct standardized microscopy, in-vitro drug-sensitivity testing and molecular testing across all three sites. Only initial data from Thailand will be presented. Patients aged 5-65 years with uncomplicated *P. falciparum* malaria, with asexual parasite density between 1,000 - 200,000/µL, no signs or symptoms of severe malaria, no other cause of febrile illness were enrolled starting in September 2013. Participants received 4 mg/kg artesunate at 0, 24, and 48 h, 15 mg/kg mefloquine at 72 h, and 10 mg/kg mefloquine at 84-96 h, with 0.5 mg/kg primaquine for transmission blocking, all under direct observation therapy. We assessed parasite density on thick/thin smears every 4 h during first 12 h after first artesunate dose and every 6 h for 72 h or until two consecutive negative smears. The parasite clearance half-life will be calculated from the parasite clearance curve. Efficacy outcome for 42 days will be assessed. Between Oct 31, 2013, and Feb 3, 2014, we assessed 52 persons suspected of malaria from four malaria clinics and hospitals in Sangkhlaburi district of Kanchanaburi province in western Thailand near Thai-Myanmar border. We screened 12 and enrolled 8 patients with *P. falciparum* malaria who met inclusion criteria. Forty cases could not be screened including 11 (27%) who previously took antimalarias including artemisinin monotherapy. Five cases had parasite clearance time more than 72 h and three cleared before 72 h [GeoMean=57.7 h (95% CI + 18)]. All eight subjects met adequate clinical and parasitological response endpoint and no recurrence was reported to date. Although no resistance to A+M detected among eight subjects in Thai-Myanmar border so far, more data to include up to 59 more subjects will be presented.

1495

PRELIMINARY RESULTS: PHASE 2 RANDOMIZED PROOF OF CONCEPT STUDY COMPARING AN INVESTIGATIONAL AMINOQUINOLINE ANTIMALARIAL (AQ-13) TO COARTEM IN ADULT MALIAN MALES WITH UNCOMPLICATED MALARIA

Ousmane A. Koita[1], Aliou Sissako[1], Sangare Lansana[1], Haiyan Deng[2], Trevor A. Thompson[2], Sahare Fongoro[1], Sounkalo Dao[1], Mamadou A. Diakite[1], Frances J. Mather[2], Boubakar Diallo[1], Asif Anwar[2], Joon Sup-Yeom[3], David M. Mushatt[2], Mark Sather[4], Donald J. Krogstad[2]

[1]University of Bamako, Bamako, Mali, [2]Tulane University Health Sciences Center, New Orleans, LA, United States, [3]Sungkyunkawan University, Seoul, Republic of Korea, [4]ScottCare, Cleveland, OH, United States

Although artemisinin-combination therapies (ACTs) are the recommended first-line treatment for uncomplicated *Plasmodium falciparum* malaria, there is increasing concern about artemisinin resistance because of prolonged parasite clearance times in southeast Asia. For this reason,

it would be helpful to have alternatives to the artemisinins that were effective against chloroquine(CQ)-resistant *P. falciparum*, were safe in human subjects and could be given orally. Our previous studies have shown aminoquinolines with modified side chains such as AQ-13 are active against CQ- and multi-resistant *P. falciparum in vitro* and in a squirrel monkey model of CQ-resistant human *P. falciparum* infection, are safe orally in human subjects and have pharmacokinetics similar to those of CQ. The preliminary results reported here are from a blinded study comparing the investigational antimalarial AQ-13 (1,750 mg over 3 days) to the current recommended first-line treatment (Coartem=artemether + lumefantrine; 480 and 2,880 mg over 3 days) for uncomplicated *P. falciparum* malaria in adult Malian males (≥18 years of age). Based on the first 33 subjects enrolled, there have been no differences in efficacy (asexual parasite clearance on or before day 7), clinical recovery (resolution of fever, chills and myalgias on or before day 3) or side effects (no serious or Grade 3 or Grade 4 adverse events) between treatment groups. Because the study is blinded, we do not know whether there are other differences between the AQ-13 and Coartem groups. However, because the second group of 33 subjects is now being enrolled, it should be possible to address those questions at the time of this presentation. The results available at this time suggest that AQ-13 alone may be as efficacious and safe as Coartem in adult Malian subjects with uncomplicated *P. falciparum* malaria.

1496

INDIVIDUAL AND HOUSEHOLD LEVEL FACTORS ASSOCIATED WITH ITN USE BETWEEN 2008 AND 2013 IN A LOW MALARIA TRANSMISSION SETTING OF SOUTHERN ZAMBIA

Jessie Pinchoff[1], Harry Hamapumbu[2], Tamaki Kobayashi[1], Jennifer Stevenson[1], Douglas E. Norris[1], Phil Thuma[2], Elizabeth Colantuoni[1], William J. Moss[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Macha Research Trust, Macha, Zambia

The prevalence of malaria has declined in parts of sub-Saharan Africa; as perceived risk decreases there are concerns that use of personal protective measures may also decrease. Determining factors that influence insecticide-treated net (ITN) ownership and use in these areas is critical to promote their continued use to achieve malaria elimination. Households in the Macha Hospital catchment area, Choma District, Southern Province, Zambia were enumerated and randomly selected using satellite imagery. Households were either visited once (cross-sectional) or every other month (longitudinal). Adults and caretakers of children were administered a survey regarding malaria-related beliefs and behaviors and a malaria rapid diagnostic test (RDT). Mosquitoes were collected in the households using light traps. Individual and household level factors associated with use among those who owned an ITN were assessed using longitudinal, multi-level regression models. Qualitative questions were tabulated to identify reasons for not owning or using an ITN. In a smaller sample of households, the association between total mosquitoes caught and ITN use was assessed to determine if culicine mosquitoes prompted use. ITN use was higher at follow-up visits (77.4%) as compared with first visits (62%) in the longitudinal cohort (p<0.0001). In the multi-level model, ITN use was 77% higher during the rainy season (OR=1.77 (95% confidence interval=1.46, 2.16)) and over twice as high after ITN distribution in June 2012 (OR=2.33 (1.21, 4.5)). Those that learned about malaria from a community health worker had 42% higher odds of using their net (OR=1.42 (1.09, 1.84)). Those that owned 3 or more nets were over twice as likely to use their ITN (OR=2.13 (1.35, 3.36)). Also, odds of ITN use was over twice as high if more than 10 culicine mosquitoes were caught in the house controlling for season and study design (OR=2.15 (1.27, 3.63)). ITN use can be sustained in low transmission settings with continued education and distributions, and may be driven in part by the presence of culicine mosquitoes.

1497

A PAN-AFRICAN HIGH-RESOLUTION SEASONAL MALARIA FORECASTING SYSTEM

Felipe J. Colón-González[1], Adrian M. Tompkins[1], Volker Ermert[2], Francesca Di Giussepe[3]

[1]The Abdus Salam International Centre for Theoretical Physics, Trieste, Italy, [2]Institute of Geophysics and Meteorology, Cologne, Germany, [3]European Centre for Medium Range Weather Forecasts, Reading, United Kingdom

Malaria transmission models are useful for understanding the epidemiology of the disease, and for the development early warning systems (EWS) in epidemic-prone regions. Dynamical models explicitly model the disease dynamics through a series of differential equations. Unlike statistical models, dynamical models are not confounded by sparse or short data records. Population density is key for determining disease occurrence, and should be incorporated in the models to effectively differentiate between urban, peri-urban, and rural malaria. Weather factors such as temperature and precipitation, are key determinants of disease niche, and should also be included in the modeling framework. Accurate predictions of weather conditions could provide useful information for targeting bespoke interventions in high-risk areas one or two months in advance. Here, we coupled a state-of-the-art dynamical malaria model that can be used at a fine spatial resolution of O(10) km, and applied over a continental scale, with two operational state-of-the-art weather prediction systems to develop a pilot malaria EWS for Africa. To our knowledge, this is the first attempt to developing a pan-African malaria EWS using state-of-the-art weather forecasts and dynamical malaria models. We determined the seasons and regions in which such a forecasting system be more valuable to decision-makers, and assessed the skill of the model. The EWS provides forecasts of malaria prevalence and intensity up to four months in advance with good skill across large regions. A further evaluation of the model using sentinel-site surveillance data demonstrates that the EWS is able to predict malaria dynamics in some target regions one to four months ahead. This findings show that the EWS could significantly help public health decision makers optimising resources, and making informed decisions about the areas and periods of high risk.

1498

SET-UP AND VALIDATION OF POST-SCREENING TOOLS FOR A NEW MALARIA TRANSMISSION-BLOCKING APPROACH BASED ON DEFORMABILITY

Papa Alioune Ndour[1], Julien Duez[1], John Holleran[2], Sylvestre Biligui[1], Liliane Cicéron[1], Nathalie Chartrel[3], Sylvie Garcia[4], Inês F. Amado[4], Harvey Tawfik[5], Bruno Le Pioufle[5], Olivier Français[5], Catherine Lavazec[6], Vicky Avery[2], Pierre A. Buffet[1]

[1]CIMI-PARIS, INSERM U1135, Paris, France, [2]Eskitis Institute, Griffith University, Brisbane, Australia, [3]AP-HP, Hôpital Pitié-Salpêtrière, Service de Parasitologie-Mycologie et Service des Maladies Infectieuses et Tropicales, Paris, France, [4]Institut Pasteur de Paris, Paris, France, [5]SATIE, CNRS UMR8029, Ecole Normale Supérieure de Cachan, Paris, France, [6]Cochin-Inserm, Paris, France

Gametocytes are the sexual forms of *Plasmodium falciparum* parasite and are essential for transmission from human to human. Unlike immature (stage I-IV) gametocytes that are stiff and sequestered, mature gametocytes (stage V) are deformable and circulate. A drug increasing their stiffness will induce their clearance by the spleen, thereby removing them from the transmission cycle. We are screening for active compounds based on their ability to induce the retention of mature gametocytes through an automated filtration process that mimics the mechanical sensing of RBC by the spleen. To validate the activity of selected compounds, we developed post-screening tools using a biomimetic microfluidic device, a simple mouse model and human spleens perfused ex-vivo. The common read-out of post-screening tools was the retention or enrichment rates of mature and immature gametocytes exposed or not to selected compounds. Typically, stage V gametocytes were pre-exposed to a

selected compound then co-infused in the microfluidic device, in mice or in the human spleens along with the same gametocyte population exposed to the solvent control. Normal RBCs, asexual ring-IRBCs and heated RBCs were used as controls. We first confirmed that unlike stage I-IV, stage V gametocytes from an *in vitro* culture were not markedly retained in microsphere-based microplate filters and in human spleen perfused ex-vivo, consistent with the hypothesis that deformability of gametocytes is a major determinant of their circulation in peripheral vessels. Using the microfluidic device, we showed that stage V exposed to a recently identified stiffening compound C were enriched to 74.9% (vs. 25.08% for unexposed controls, p=0.0001 paired t test) in narrow 2 µm-wide spaces mimicking inter-endothelial slits in the spleen. In macrophage-depleted C57 Bl/6 mice, immature gametocytes (10 mice) and heated RBCs (4 mice) were cleared by 86% or 75% in 3 hours, respectively. By contrast, a majority of mature gametocytes (5 mice) or normal RBCs (4 mice) were still circulating 3 hours after infusion (Retention rates: 44% and 30%, respectively (p=0.0058, p=0.0002). Similar results were observed in human spleens. Mature circulating gametocytes can be stiffened to induce their mechanical retention, thereby interrupting transmission. The stiffening effect can now be validated in a biomimetic microfluidic device and in a simple rodent model as a prerequisite before further development.

1499

THE USE OF RESPONDENT DRIVEN SAMPLING METHODS TO IDENTIFY MALARIA PREVENTION KNOWLEDGE AND BEHAVIORS BY MIGRANT AND MOBILE POPULATIONS IN WESTERN CAMBODIA

John Hustedt[1], Chandary Rang[1], Sophal Uth[1], Sara Canavati[1], John Macom[1], Po Ly[2], Siv Sovannaroth[2], Arantxa Roca-Feltrer[1], Tarekegn Abeku[1], Sylvia Meek[1]

[1]Malaria Consortium, Phnom Penh, Cambodia, [2]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia

Mobile and migrant populations (MMPs) along the Thai-Cambodian border are at high-risk for malaria infection and have been found with artermisinin resistant parasites. However, the mobile nature of this population makes it difficult to adequately measure malaria infection and risk behaviors, which is vital as we move to elimination in the region. Utilizing respondent driven sampling methods, MMPs residing within two villages in Palin province (Pang Rolim and Sala Krau) were recruited in two independent rounds of sampling (602 in 2013 and 604 in 2014). All responses were adjusted for network size and recruitment patterns allowing for calculation of population-adjusted statistics. While the prevalence of *Plasmodium vivax* is estimated to be 0.2% among the general population, this study found 2.0% and 1.3% of MMPs in these networks to be infected with *P. vivax* in 2013 and 2014 respectively, and an absence of *P. falciparum*. Most respondents from Pang Rolim, from both rounds, identified having seen malaria messages within the previous three months (99.7%, 95% CI: 97.6-100 in 2013 and 99.0%, 95% CI: 95.9-99.8 in 2014). However, in Sala Krau, the percentage of respondents answering similarly decreased from 97.0% (95% CI: 94.1-98.4) in 2013 to 59.1% (95% CI: 51.3-66.4) in 2014. While knowledge related to malaria transmission, symptoms and prevention increased noticeably in Pang Rolim, similar knowledge remained low in Sala Krau across both rounds. Furthermore, while the percentage of respondents from Pang Rolim who didn't use a net the previous night remained the same across both rounds (2.4%), there was a slight increase in non-users in Sala Krau from 6.1% (95% CI: 0.9-6.7) to 9.5% (95% CI: 5.3-16.4). These findings correlate with the fact that there were increased efforts on malaria prevention in Pang Rolim (eg. concerts and videos with prevention messaging) and not in Sala Krau; suggesting that as MMPs change frequently there is a need for sustained public health efforts to reach this population, especially within an elimination context.

1500

IMPLEMENTING ENHANCED HIGH-RESOLUTION SURVEILLANCE USING SPATIAL DECISION SUPPORT SYSTEMS TO GUIDE TARGETED RAPID RESPONSE IN MULTI-DRUG RESISTANT AREAS OF VIETNAM

Gerard C. Kelly[1], Colin Ohrt[1], Hieu M. Truong[2], Hien T. Nguyen[3], Nicholas Martin[4], Xa X. Nguyen[2], Quy Anh Nguyen[2], Roland Gosling[1], Jennifer Wegbreit[1], Duong T. Tran[2], Duong T. Tran[2], Thang D. Ngo[2]

[1]University of California San Francisco, San Francisco, CA, United States, [2]National Institute of Malariology, Parasitology and Entomology, Hanoi, Vietnam, [3]U.S. Embassy, Hanoi, Vietnam, [4]Naval Medical Research Center-Asia, Singapore, Singapore

Emerging artemisinin resistant malaria in the Greater Mekong Subregion (GMS) has important implications for public health. A project was established to research, develop and implement enhanced surveillance and targeted appropriate intervention measures to stop the spread of multi-drug resistant malaria through elimination of the disease in the region. The aims of this project are to pilot a spatial decision support system (SDSS) approach to conduct high-resolution surveillance to guide swift and targeted responses. Pilot sites were established in selected communes in Vietnam with associated customised SDSS developed. Publically available topographic geographic information system data were uploaded into the SDSS to provide baseline information. Household and forest transmission location data were located and enumerated through field-based geographical reconnaissance using handheld computers. Passively detected malaria cases were geo-referenced to the suspected transmission location sites upon diagnosis. Using case location data in the SDSS, active transmission foci were automatically classified and response areas-of-interest (AOI) generated. Supporting data (including population, location and number of sleeping locations within the AOI) were automatically produced in the SDSS and sent to village health workers and district level units to mobilize appropriate responses. Complete pilot data for presentation are expected in September 2014. This new approach utilizing novel geo-spatial tools to support targeted, appropriate and aggressive response measures to support malaria elimination in areas of global significance will be presented.

1501

RESTRATIFICATION OF MALARIA EPIDEMIOLOGY IN VIETNAM FOR MORE EFFECTIVE APPLICATION OF LIMITED RESOURCES

Thang D. Ngo[1], Hung X. Le[1], Hung M. Nguyen[1], Thieu Q. Nguyen[1], Trung D. Ho[1], Xa X. Nguyen[1], Anh Q. Nguyen[1], Ha S. Dinh[1], Roland Gosling[2], Bruce Lee[3], Hien T. Nguyen[4], Gerard C. Kelly[3], Nicholas Martin[5], Colin Ohrt[3], Duong T. Tran[1], The NIMPE Advisory Groups[1]

[1]National Institute of Malariology, Parasitology and Entomology, Hanoi, Viet Nam, [2]University of California San Francisco, San Francisco, CA, United States, [3]U.S. Embassy, Hanoi, Viet Nam, [4]Naval Medical Research Centre - Asia, Singapore, Singapore

The National Malaria Control Program in Vietnam is updating malaria epidemiology in order to more effectively apply limited malaria diagnosis, prevention and treatment resources. The most recent prior restratification was conducted in 2009. This on-going 2014 restratificaton effort (2009-2013 data) is using the same methods of 2009 to collect all malaria case data to the commune (county) level. Indicators for classification are based on the average number of confirmed cases per 1000 population over the 5 year period, the presence of at least one of the three malaria vectors, socioeconomic disadvantaged or border commune, poor health system, drug resistant parasites, chemically resistant mosquitoes, and migratory populations. Each indicator has a score, with the sum of the scores used to define the level of endemicity and priority for interventions. This score will be used to characterize each commune into one of five zones (no