establish P.f. W2 as our standard laboratory clone for HRP2 (Histidine-rich protein 2)-ELISA based drug sensitivity assay.

## 180

### MIXED MALARIA: FICTION OR REALISM?

**Ana C. Giraldo**

*Universidad de Antioquia, Medellin, Colombia*

Malaria is a serious public health problem in Colombia. In the Pacific region predominates *Plasmodium falciparum*, and the rest of the country *Plasmodium vivax* in a ratio of 8:1 *P.vivax* / *P. falciparum*. However, the increasing frequency of mixed infections *P. falciparum* + *P. vivax* is a phenomenon by studying. One of the reasons for this phenomenon is the coexistence of *P. falciparum* and *P. vivax* in the country, which could explain the high numbers of complication and death that occur in Colombia. Historically, these complications have been associated with *P. falciparum*, and lately have been reported cases of malaria complicated by *P.vivax* in malarious areas. Group malaria at the University of Antioquia has established that many of these complications due to mixed malaria (P.falciparum-P.vivax), which could underestimate by inexperience in the diagnosis of malaria. This work shows the increase of mixed Malaria in Colombia from 2004 to 2008, and highlights the seriousness of the problem, in order to design preventive measures. Databases consulted included the Colombia Ministry of Social Protection and File Malaria Clinical Group / University of Antioquia. SIVIGLA reported 447.684 cases distributed as follows: *P. vivax* 67.851 (59.83%), *P. falciparum* 173.234 (38.69%), malaria mixed 6.530 (1.45%), *P. malariae* 69 (0.015%). The Malaria Group reported 617 malaria cases positive for malaria, comprising: *P. vivax* 387 (62.72%), *P. falciparum* 149 (24.14%), mixed malaria 81 (13.72%). Most of these patients came from Antioquia, Córdoba and Chocó. The discrepancy between the two databases possibly due to: 1) malaria mixed is a growing phenomenon underdiagnosed in Colombia; or 2) the misdiagnosis of malaria. So, it is possible to think that is common the miss therapy antimalarial.

## 181

### CHANGING PATTERN OF CHLOROQUINE-SUSCEPTIBILITY OF *PLASMODIUM VIVAX* IN THE REPUBLIC OF KOREA

**Jae-Won Park**[1], Gyo Jun[1], Joon-Sup Yeom[2], Hyeong-Seok Lim[3]

*[1]Graduate School of Medicine, Gachon University of Medicine and Science, Incheon, Republic of Korea, [2]Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea, [3]Ulsan University College of Medicine, Seoul, Republic of Korea*

The total number of vivax malaria patients in the Republic of Korea (ROK) and North Korea is estimated to be close to a million cases since its re-emergence in 1993. To cope with this situation, the ROK Army has performed chemoprophylaxis with hydroxychloroquine (HCQ) and primaquine since 1997. The cumulative number of ROK Army soldiers given chemoprophylaxis exceeded 1.6 million by 2008. The extensive chemoprophylaxis contributed to prevent a rapid increase of malaria patients in ROK Army, but raised the possibility of the occurrence of chloroquine (CQ)-resistant *Plasmodium vivax* strains. Prophylaxis has consistently failed in many cases despite attainment of sufficiently high plasma concentrations of HCQ. Moreover, the length of time required for the elimination of *P. vivax* from patients' blood by HCQ treatment has delayed in the current decade. Recently, CQ-resistance was confirmed in two patients, which was the first report of CQ-resistant *P. vivax* in a temperate region of Asia. Continuous surveillance is warranted to monitor the change in CQ-susceptibility and CQ-resistance frequency of *P. vivax* in ROK.

## 182

### COMPARATIVE STUDY OF *PLASMODIUM FALCIPARUM* GROWTH IN SERUM-FREE MEDIA AND EXPRESSION LEVELS OF *PFCRT* GENE AFTER EXPOSURE TO DRUG

**Bethel Kwansa-Bentum**[1], Kei Kitamura[1], William K. Anyan[1], Takashi Kumagai[1], Shinji Izumiyama[2], Hiroko Asahi[2], Michael D. Wilson[3], Nobuo Ohta[1]

*[1]Tokyo Medical and Dental University, Tokyo, Japan, [2]National Institute of Infectious Diseases, Tokyo, Japan, [3]Noguchi Memorial Institute for Medical Research, Accra, Ghana*

Malaria continues to be a major global health emergency, with increasing demand for new drugs with alternate targets. It is known that the principal causative agent, *Plasmodium falciparum* requires some factors in the human serum (HS) to develop, albeit the role of HS in growth of the parasite is not known. It is possible that HS-based culture might give unstable results because of inter-lot heterogeneity in HS. In this study, in-vitro assessment of *P. falciparum* parasites using serum free culture media (Growth-promoting fraction; Albumax II; Chemically Defined Medium) and HS were carried out. Four growth assessment techniques (Giemsa staining; Flow cytometry; pLDH; HRP 2) were employed after the parasite was exposed to drugs (chloroquine; artemether). Dose-response curves were used to determine the 50% inhibitory concentration ($IC_{50}$) values. The relationships between parasite's response to chemotherapy and expression levels of genes, such as *Pfcrt* and *Pfmdr* that are implicated in chloroquine resistance were also studied. Here, we sort to throw more light in understanding the molecular basis of resistance in malaria parasite.

## 183

### PREVALENCE OF MUTATIONS IN *PFCRT, PFDHFR* AND *PFDHFR* GENES CONFERRING DRUG RESISTANCE IN *PLASMODIUM FALCIPARUM* MALARIA ISOLATES FROM SOUTHERN PAKISTAN

**Mohammad A. Beg**[1], Najia K. Ghanchi[1], Johan Ursing[2], Sana Jafri[1], Andreas Martensson[2]

*[1]Aga Khan University, Karachi, Pakistan, [2]Karolinska Institute, Stockholm, Sweden*

Resistance to antifolate chemotherapy is a significant problem in malaria patients in Pakistan where Sulfadoxine-pyrimethamine (SP) is recommended as a partner drug of ACTs for the treatment of *Plasmodium falciparum* malaria. This region has empirical treatment failure of *P. falciparum* to Chloroquine (CQ) and SP, making this readily available option defunct. We present preliminary results on prevalence of SP and CQ resistance in our region using molecular tools. A total of 240 patients infected with *P. falciparum* presenting at Aga Khan University clinical laboratories during 2005-2007 were included in our study. The prevalence of mutations at *pfcrt* gene codonK76T, *pfmdr1* N86Y, *pfdhfr* C50R, N51I, C59R, S108N, I164L and A436S, G437A and E540K were genotyped by multiplex PCR/RFLP and pfdhfr by sequence analysis of amplified product in *P. falciparum* isolates from Southern, Pakistan. Single nucleotide polymorphism (SNPs) in *pfcrt*, *pfdhps* and *pfdhfr* genes associated with resistance to CQ and SP were observed. Prevalence of 108N was 99%, 59R 96% and 51I 7.6% conferring resistance to SP position 50 and 164 remains unchanged, whereas 437G in *pfdhps* was observed in 51% and 540E in 1% of the isolates. Almost 92% (223/242) of *P. falciparum* isolates carried the 76T and markedly few isolates had 76K polymorphism. In conclusion, we confirm the existence of mutations conferring resistance to CQ and SP in *P. falciparum* population using modern molecular tools. The level of mutations associated with resistance suggests that SP as a partner drug with ACTs has no role in *P. falciparum* infections. In addition CQ should not be used for *P. falciparum* infections. Therefore we expect high levels of treatment failures with these drugs and suggest removal from the current treatment strategy. This data may be used in helping to formulate a rational drug policy.

## 184

### CAN THE SPATIAL DISTRIBUTION OF DUFFY NEGATIVITY EXPLAIN *PLASMODIUM VIVAX* ABSENCE IN AFRICA?

**Rosalind E. Howes**, Fred B. Piel, Carlos A. Guerra, Peter W. Gething, Anand P. Patil, Simon I. Hay

*Spatial Ecology and Epidemiology Group, Department of Zoology, University of Oxford, Oxford, United Kingdom*

The striking absence of *Plasmodium vivax* from large swathes of tropical Africa has been postulated to be due to high frequencies of Duffy negative blood groups in human populations. Molecular studies of the Duffy Antigen Chemokine Receptor (DARC) lend support to this hypothesis, as the antigen has been demonstrated to be necessary for *P. vivax* erythrocyte invasion, rendering Duffy negative individuals immune to *P. vivax*. The spatial extent to which Duffy negativity may explain the absence of *P. vivax* in Africa has never been considered rigorously. The main focus of the present research is the development of the first contemporary, evidence-based and statistically-robust map of Duffy negativity in Africa. This was achieved following assembly of a comprehensive database of geographically-specific community blood sample surveys, giving Duffy antigen genotypes or phenotypes found. The predictive surface map was derived from analysis of the dataset using state-of-the-art geostatistical models. To test the extent to which the mutant Duffy negative phenotype is protecting the African population from *P. vivax* infection, a map of the expected distribution of *P. vivax* was generated based upon climate suitability for parasite development and survival of its *Anopheles* vectors, combined with fine-resolution population distribution data. Areas of non-overlap between Duffy negative populations and zones of predicted potential transmission were examined alongside the contemporary observed *P. vivax* distribution. The series of continental maps produced are presented, with discussions focusing on regional-level comparisons, notably the observed differences between Western-Central regions and East Africa.

## 185

### SPATIAL PREDICTION OF *PLASMODIUM FALCIPARUM* PREVALENCE IN INDONESIA IN 2008

**Iqbal Elyazar**[1], Hanifah Rogayah[2], Pete Gething[3], Anand Patil[3], Carlos Guerra[3], Desak Wismarini[2], Rita Kusriastuti[4], Simon Hay[3], Kevin Baird[1]

*[1]Eijkman-Oxford Clinical Research Unit, Jakarta, Indonesia, [2]National Communicable Disease Center, Sub-directorate of Malaria, Ministry of Health, Jakarta, Indonesia, [3]Spatial Ecology and Epidemiology Group, Tinbergen Building, Department of Zoology, University of Oxford, Oxford, United Kingdom, [4]National Communicable Disease Center, Ministry of Health, Jakarta, Indonesia*

Nationwide surveillance of malaria prevalence is key to monitoring the changing epidemiology of malaria in countries with a willingness to scale up coverage of malaria interventions. The epidemiology of malaria in Indonesia has been broadly described, no reliable contemporary distribution map of the prevalence of *Plasmodium falciparum* in Indonesia has been developed using robust geospatial modeling technology. An exhaustive search and assembly process for a national database of *Plasmodium falciparum* parasite prevalence surveys is described. The database held over 2,000 spatially independent parasite rate estimates from community surveys conducted in Indonesia since 1985. Bayesian geostatistical models were used to predict continuous maps of *Plasmodium falciparum* prevalence across Indonesia. The models were used parasite prevalence survey data, climatic variables and other covariates. The models were also used to define the uncertainty associated with the predictions. These results are summarized across the archipelago and the implications for malaria control and elimination elaborated.

## 186

### EFFECTS OF A LOW-INFECTIVITY RESERVOIR IN MALARIA ENDEMIC POPULATIONS ON TRANSMISSION AND LONG TERM CONTROL

**Stephan Karl**[1], Peter A. Zimmerman[2], Tim G. St.-Pierre[1], David Gurarie[2]

*[1]The University of Western Australia, Perth, Australia, [2]Case Western Reserve University, Cleveland, OH, United States*

Modern diagnostic tools, like magnetic deposition microscopy (MDM) and PCR-based methods allow better detection of infectious *Plasmodium* gametocytes in human populations. Thus infectivity to mosquitoes as determined by gametocyte density, a critical factor for understanding malaria transmission potential, can be better evaluated. Field application of these techniques in recent studies has identified a substantial pool of human hosts with gametocytemia below the threshold of detection, and prevalence significantly higher than estimated by conventional blood smear light microscopy (LM). The resulting challenge is to determine how such low level infective strata affect the transmission and control of Plasmodium infection in endemic areas. One way to address questions arising is through mathematical modeling, the origin of which dates back to the classical works by Ross, Macdonald and others. Here we developed refined versions of the Ross-Macdonald model that introduces multiple infective strata, which includes the low gametocytemia reservoir. We calibrated the models by using field-based data from studies in Papua New Guinea. Using these models we conducted several numeric experiments to assess the effect of low gametocytemia on malaria transmission and control. We looked in particular, at the long term impact of a malaria control campaign based on insecticide treated bed nets (ITN; presently considered the most efficient way to prevent transmission). The analysis showed the following significant differences. Ross-Macdonald systems including high and medium infectivity, but without the low gametocytemia reservoir, predicted "finite eradication times" after introduction of an ITN based control campaign. Our extended model including the low gametocytemia reservoir predicted stable transmission to persist. The models developed here also provide significantly different estimates for the level and duration of control to achieve eradication. Our findings suggest that it will be important to apply high sensitivity diagnosis of gametocytemia to achieve goals of malaria eradication.

## 187

### ARE FISH FARMING ACTIVITIES CONTRIBUTING TO MALARIA TRANSMISSION IN THE PERUVIAN AMAZON?

**Mathieu Maheu-Giroux**[1], Martin Casapia[2], Veronica E. Soto-Calle[3], Lea Berrang Ford[1], David L. Buckeridge[1], Oliver T. Coomes[1], Theresa W. Gyorkos[1]

*[1]McGill University, Montréal, QC, Canada, [2]Asociación Civil Selva Amazonica, Iquitos, Peru, [3]Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium*

Malaria re-emergence in the Amazon Basin of South America is thought to be associated with the socio-environmental changes that have occurred regionally since the 1980s. In the Department of Loreto (Peru), one such modification is the development of fish farming, which has become an important economic activity, but is suspected of contributing to increased malaria transmission. This hypothesis, is subject controversial, however, because the principal malaria vector in the region (Anopheles darlingi) is generally considered sylvatic and riverine and its preferred breeding habitat is characterized by clean, partially-shaded, slow-moving waters. Such breeding conditions are not found in fish ponds, which typically hold open stagnant waters with high concentrations of organic matter. To test this hypothesis, we quantified the impact of fish ponds on malaria incidence by conducting a cohort study. Data on fish pond density around households and malaria incidence were collected retrospectively for a 30-month period (2006-2008) in eight rural communities south of Iquitos, the capital of Loreto. Malaria incidence was ascertained by using the

malaria registries of the government's local health post, which consist of data from both active and passive surveillance (238 cases). Fish pond density was measured using an interpreted satellite image and information on potential confounders (age, sex, SES, house characteristics, occupation, etc.) was collected through interviewer-administered questionnaires (914 subjects in 234 households). The association between fish pond density and malaria incidence was quantified using Generalized Estimating Equations, controlling for confounders. Fish pond density, measured by summing the length in kilometre of the pond/land interface in a 500-meter buffer around households, was strongly associated with malaria incidence (adjusted OR 1.24, 95% CI 1.12-1.39). Our findings have important public health implications and poses dilemmas for this poverty stricken region where fish ponds provide a much needed source of income and nutrition.

## 188

### ARE RDTS COST-EFFECTIVE AND SAFE? A SYSTEMATIC REVIEW OF ACCURACY IN MALARIA DIAGNOSIS

**M. S. Newman**, Latha Rajan

*Tulane University, New Orleans, LA, United States*

Malaria diagnosis now includes rapid diagnostic tests (RDTs). The WHO has published recommendations for RDT use and cost-effectiveness analyses have been done. These analyses recommend RDTs should have ≥95% sensitivity. Specificity should approximate 80%. To examine whether RDTs in current practice meet these guidelines, we conducted a systematic review of studies of the diagnostic accuracy of RDTs. Using MEDLINE, EMBASE and CINAHL databases (1990-December 31, 2008), we compiled original studies on the diagnostic accuracy of RDTs for malaria induced by any species of Plasmodium in any part of the world. We extracted sensitivity, specificity, parasite species, test type, year of the study, and location of the study and eliminated studies that were not consecutive, prospective and blinded and those that were solely concerned with malaria in pregnancy. We screened 1,908 citations and include 74 studies here. We analyze sensitivities, specificities, and heterogeneity of these studies. We compare these to the parameters set by various cost-effectiveness studies to draw conclusions about the usefulness of RDTs. We find a wide range of values for sensitivity (2.90% to 100%) and specificity (52.00% to 100%), and establish the large majority (95.6%) of tests have specificity ≥80%. However, only 25.6% had sensitivity ≥95%, and roughly one-quarter had sensitivities <80%. We find an overall I2 value of 94.9% -- showing that, overall, a high amount of heterogeneity in study design, sufficient that weighted average of sensitivities and specificities were not meaningful. In conclusion, we establish that specificity is sufficient in RDTs but sensitivity is inconsistent and often low. Although RDTs are useful where presumptive diagnosis would otherwise be the only system available, it might be neither useful to replace microscopy nor superior to a careful clinical algorithm. However, the heterogeneity of studies implies the conclusions we draw from this meta-analysis are tentative. Data tends to be unreliable, leaving the question of RDT accuracy unanswered.

## 189

### DETERMINANTS OF USE OF MALARIA PREVENTION STRATEGIES DURING PREGNANCY IN UGANDA

**Laura Sangare**[1], Noel Weiss[1], Sarah Staedke[2], Steven Kiwuwa[3], Diane Martin[1], Thomas Lumley[1], Andreas Stergachis[1]

*[1]University of Washington, Seattle, WA, United States, [2]London School of Hygiene and Tropical Medicine, London, United Kingdom, [3]Makerere University, Kampala, Uganda*

Maternal malaria is associated with serious adverse pregnancy outcomes. Established means of preventing malaria during pregnancy include intermittent presumptive therapy (IPTp) with sulfadoxine/pyrimethamine (SP); and sleeping under an insecticide treated net (ITN). We sought to identify determinants of preventive use of SP and ITNs during pregnancy in a cohort of post-partum women in Uganda. Utilizing a cross-sectional study design, we interviewed a random sample of 500 women living in rural and peri-urban villages of Uganda who had been pregnant in the past year. Determinants of SP and ITN use included socio-cultural, individual-level, and healthcare facility-level factors. Of the 500 participants, 99% attended at least 1 antenatal care (ANC) visit, of which, 68% were offered SP at least once for prevention. Among women who were offered SP, uptake was 98%. However, 152 women (31%) who attended ANC were never offered SP, 92% of whom reported 2 or more ANC visits. When asked about factors which influence their decision to use SP, being told by a doctor, nurse, or midwife was more influential among SP users ($p<0.001$), whereas, among non-users being offered SP for free was the most influential factor ($p=0.006$). Preventive SP users were more likely to believe that SP could commonly cause miscarriage, Steven Johnson's syndrome, or harm the fetus compared to non-users ($p<0.0001$). The single largest predictor of preventive SP use was being offered SP during ANC ($p<0.0001$). ITNs were owned by 384 women, of whom 71% were compliant (i.e reported they always slept under the ITN during pregnancy). No differences were found between compliant and non-compliant ITN users regarding receipt of ITN education from a doctor, nurse, or midwife, or number of ANC visits. These findings suggest that women will use SP if provided at ANC. Thus to increase uptake of IPTp the primary focus should be on improving access to SP. However, ownership of an ITN is not sufficient for 100% compliance of ITN use. More evidence is needed to identify effective strategies to improve compliance of ITN use during pregnancy.

## 190

### RISK MAPPING TO SUPPORT MALARIA ELIMINATION IN VANUATU

**Heidi Reid**[1], Archie Clements[1], Andrew Vallely[1], George Taleo[2]

*[1]University of Queensland, Brisbane, Australia, [2]National Vector Borne Disease Control Program, Port Vila, Vanuatu*

The advancement of geographical information systems and spatial statistics has allowed the mapping of the bounds of malaria transmission and variation in malaria prevalence within these bounds. As the objective of a malaria program shifts from control to elimination and strategies become more focussed and resource intensive, there is more urgent need to understand malaria dynamics at a finer geographic level. However, spatial risk profiling of malaria at a small geographical scale has only been explored in only a handful of cases. The Ministry of Health in Vanuatu, plans to implement a malaria elimination program on Tanna Island, the most southern and eastern limit of malaria in the South West Pacific. A 2008 parasitological survey on Tanna found an overall prevalence rate of 1.9% (N=5239) with *Plasmodium falciparum* accounting for 16% of infections and *Plasmodium vivax* 74%. Using these findings, locations of villages were geo-referenced and spatial autocorrelation was assessed using a variogram. Regression analysis was conducted on individual-level covariates including age, sex and bednet use. Environmental covariates including normalised difference vegetation index (NDVI) and thermal infrared (TIR) were assessed for association with malaria prevalence. A Bayesian geostatistical model was developed to predict malaria risk, and associated uncertainty across the island. Regression analysis showed no significant association with NDVI, TIR, age, sex or bednet use of the child. However, significant spatial autocorrelation was detected and spatial prediction showed a clustered spatial pattern with clusters of high-risk communities present in the far northern and southern reaches of the island. In conclusion, our findings are consistent with other studies suggesting that environmental factors are less important when analysing malaria prevalence at a finer geographical scale, and that other, currently unidentified factors operate to determine small-scale spatial variation. This study will be consolidated with an upcoming study using serological markers to determine long-term trends in transmission across the island and identification, at the level of the household, of areas at high risk of malaria. This risk mapping study of malaria on Tanna Island in Vanuatu represents the first application of this tool in an elimination campaign.

## 191

### SEQUENCE POLYMORPHISMS OF THE *PLASMODIUM FALCIPARUM* MEROZOITE SURFACE PROTEIN-2 (*MSP-2*) REVEAL A HIGHER COMPLEXITY OF INFECTION IN MALARIA-INFECTED CHILDREN

**Segun I. Oyedeji**[1], Henrietta O. Awobode[2], Jürgen F. Kun[3]

*[1]Bells University of Technology, Ota, Ogun State, Nigeria, [2]University of Ibadan, Ibadan, Nigeria, [3]Institute of Tropical Medicine, Tübingen, Germany*

*Plasmodium falciparum*, the species of malaria parasite that causes life threatening forms of the disease is antigenically diverse, aiding escape from host immune defenses and posing considerable obstacles to vaccine design. MSP-2 is a secreted polymorphic antigen associated with the asexual blood-stages and is encoded by highly divergent alleles grouped into two dimorphic families, FC27 and 3D7. Traditionally, complexity of infection is determined by gel electrophoresis of PCR product. In this study, we assessed the diversity of *P. falciparum* infections in children with uncomplicated malaria (UM) and non-symptomatic infections (NS) by *MSP-2* genotyping on gel electrophoresis and subsequently, by direct sequencing of PCR product. DNA was extracted from blood spot on filter paper using the QIAmp® DNA Mini Kit. Parasites were typed using sequence specific primers. Purified PCR products of samples showing single band were sequenced using Big Dye terminator reaction mix and separated by capillary electrophoresis in ABI PRISM® 3100. A total of 34 and 36 distinct MSP-2 alleles were respectively found in children with UM and NS. However, when single bands were sequenced, the number increased to 43 and 51 for UM and NS respectively. The odds of having a multiple infection (defined as the minimum number of distinct *P. falciparum* genotype) was greater in children with non-symptomatic infections compared to those with uncomplicated malaria (OR 1.65, 95% CI 1.26- 2.14, *P*= 0.014). Distribution of 3D7 alleles were significantly higher in children with UM compared to NS (*P* = 0.029). Isolates in this region are genetically very diverse. Detection of a single band on agarose gel electrophoresis does not necessarily imply identical allele or genotype. This result has great implications for intervention studies that rely exclusively on this technique to discriminate between strains and especially in distinguishing recrudescence from new infections.

## 192

### THE CHANGING PATTERNS OF MALARIA ADMISSIONS SINCE 1999 AT 18 HOSPITALS ACROSS KENYA

**Emelda A. Okiro**, Julliette Mutheu, Pete W. Gething, Elizabeth Juma, Robert W. Snow

*Kenya Medical Research Institute-Wellcome Trust Research Programme, Nairobi, Kenya*

The last few years have witnessed a rapid scaling up of key malaria interventions in a number of African countries, most notably insecticide treated nets (ITN) and improved therapeutics such as Artemisinin based combination therapy (ACT). However, there is only limited information on the health impact of expanded coverage of these interventions in Kenya. Paediatric admission data were assembled over 9 years from 18 district hospital settings in Kenya. Study hospitals were selected to reflect the diverse malaria ecologies typical of Kenya. Trends in monthly malaria admissions between January 1999 and July 2008 were analysed using several time-series models controlling for covariates related to climate and service use to establish whether changes in admissions can be attributed to expanded coverage of ITNs and ACT. There was evidence that the hospital burden for children with a primary admission diagnosis of malaria has declined over the interval at several sites; notably in areas where starting endemicity was low, moderate or acutely seasonal (the coast, semi arid and highland areas). Conversely, the overall malaria pediatric admission burden remained relatively constant or increased slightly over the observation period at most sites along Lake Victoria and in western Kenya where transmission intensity was higher. These trends remained after controlling for rainfall and population growth. Covariate analysis of ITN and ACT access and coverage showed a strong correspondence with temporal changes in admissions where declines were observed. In conclusion, this study provides evidence of significant within country heterogeneity in temporal trends of malaria disease burden corresponding to differences in malaria transmission intensity and intervention coverage.

## 193

### EXPRESSION OF RECOMBINANT PROTEINS FROM TROPHOZOITES AND MEROZOITES OF *PLASMODIUM FALCIPARUM* ISOLATES FROM BRAZILIAN AMAZON AND IMMUNE RESPONSE ANALYSIS

**Márcia M. Medeiros**, Catarina Castineiras, Gerhard Wunderlich

*University of Sao Paulo, São Paulo, Brazil*

The effective humoral immune response against the human malaria parasite *Plasmodium falciparum* is believed to be directed against parasite-encoded antigens at the infected erythrocyte surface and merozoite surface antigens although it is largely unclear which antigens are important targets and which are recognized by simple exposure. In order to define an effective humoral immune response, we have cloned and expressed recombinant proteins from a number of IRBC or merozoite surface-exposed antigens such as SURFINs, several merozoite antigens (MSP-1 to MSP-9), erythrocyte binding antigens (EBA-140, EBA-175, EBA-181) and apical merozoite antigen 1 (AMA-1), from *Plasmodium falciparum* field isolates of Brazilian Amazon. Sequencing data of the amplified part of surf genes exon 1, the putatively variable region of the 10 genes, showed a high level of similarity between field isolates, FCR3 and 3D7 strains, however in some field samples surf genes seemed deleted as shown by either PCR or pulse field gel electrophoresis experiments pointing to a deletion of the whole gene or part of the respective surf gene. We then specifically addressed the differencial recognition of SURFIN-related GST-fusion proteins in ELISA. Sera of a pool of i) symptomatic and ii) asymptomatic patients from a seasonal malaria-endemic area of the Brazilian Amazon were utilized as primary antibodies and the results showed a different recognition profile in these groups related to rSURFINs using rMSP3 (3D7-like) as control antigen. While the MSP3 antigen, as expected, was stronger recognized by the asymptomatic group sera, rSURFINs were only poorly recognized by either group. The ELISAs with the remaining recombinant antigens are in progress and results and results will be discussed.This work is supported by FAPESP.

## 194

### MALARIA RELATED WITH SOCIAL CHARACTERISTICS, SYMPTOMS AND PARASITE DENSITY, TIERRALTA, CORDOBA, COLOMBIA

**Maria F. Yasnot**[1], Yicel Alvarez[1], Maria Angelica Ramos[1], Villa Keny[1], Gustavo Enrique Quintero[2]

*[1]Universidad de Cordoba, Monteria, Colombia, [2]Grupo de Investigaciones Microbiologicas y Biomedicas de Córdoba, Monteria, Colombia*

In malaria, as in the others infections there risk factors that considerate more associate or to depend of the parasite, others of the human host and others of the host-parasite environment. Belonging the risk factors more related with the parasite are include the specie, genetic variations and parasite density. The risk factors more related with host are gender and age, the nutritional and the immunity, occupation, behavior of relationship social and natural environment. Is important to consider clinical rules base on parasite density and symptoms should be adjusted accord to transmission and geographic specific area. The proposal of this study was to describe relationship between density parasite, symptoms and socio demographic characteristics. Thick and thin smears were carry out from 115 patients that go to Secretaria de Salud Municipal de Tierralta, considerations and selection criteria were considerate. Symptoms and socio demographics data were collected by personal questions. Of 115 patients, 35,65% was women and 64,34% was men, 65 and 19,920

parasite/µL were range of parasite density to *P. vivax* and 68 and 19,630 parasite/µL were range of parasite density to *Plasmodium falciparum.* Fever, headache, chills and discomfort were predominant symptoms in women and men. Eighteen until thirty five years old were the range of the patients that present more number of cases of *P. vivax.* In this study was not observed relationship between parasite density and number of previous malarias. 100 patient referred have had until 10 previous malarias, 78 of them with low parasite density (minor of 5000 parasite/µL). Furthermore, was not observed direct relationship between the occupation and the number of times that the person has presented malaria in its life. The majority of patients with infection by *P. vivax* or *P. falciparum*, presented between 5 and 7 symptoms, 63% and 67% respectively, with parasite densities below 5,000 parasite/µL, and no direct relationship was observed between parasite density and the number of symptoms by patient. Almost populations were mestizo etnia. Study Population doesn't show relationship between density parasite, symptoms and socio demographic characteristics.

## 195

### TOWARD MALARIA EARLY WARNING IN AFGHANISTAN USING REMOTE SENSING

**Richard Kiang**[1], Najibullah Safi[2], Farida Adimi[1], Radina Soebiyanto[1]

*[1]NASA Goddard Space Flight Center, Greenbelt, MD, United States, [2]National Malaria and Leishmaniasis Control Program, Ministry of Public Health, Kabul, Afghanistan*

Malaria is one of the major public health problems in Afghanistan, ranked as the second highest burden of malaria in WHO EMRO and the fourth worldwide outside Africa. Malaria in Afghanistan occurs at altitudes below 2,000m and is most prevalent in snow-fed river valleys and rice growing areas. Transmission is seasonal from June to November, and negligible between December and April. Based on historical data on malaria morbidity, climate and attitude, the country is divided into three risk strata - high, low, very low or malaria free provinces. The provinces in each stratum do not have homogeneous malaria risk. Environmental variables - including precipitation, temperature, elevation and vegetation index - are known to influence malaria transmission. In order to provide an early warning system, we have previously developed neural network methods to predict malaria cases in Southeast Asia using environmental variables, and we have applied these methods to malaria epidemiology in Afghanistan. The 2004-2007 provincial malaria data were used in this study. Ten provinces with more complete epidemiological record were selected for training and prediction. The environmental data were extracted from NASA satellite measurements, including those from MODIS, TRMM and SRTM. Our hindcasts show a reasonably well malaria prediction in provinces with more established public health support and stable malaria transmission. The actual incidence in provinces with increasing public health support and awareness - that helps curtail malaria transmission - may be lower than what is warranted by meteorological and environmental conditions in the model. On the other hand, where refugees have returned or substantial population movements have occurred may see an enhanced malaria transmission, leading to under prediction. The sensitivity of malaria incidence with respect to each meteorological and environmental parameter will be discussed. The predictive and early warning capabilities shown here supports WHO goal.

## 196

### IS FEVER A GOOD SCREENING TEST FOR MALARIA SURVEYS IN MELANESIA?

**G. Dennis Shanks**

*Australian Army Malaria Institute, Enoggera, Australia*

Malaria surveys attempt to quickly estimate the malaria burden in an area. In endemic areas it is widely observed that older children and adults tolerate malaria parasites without symptoms unlike younger children. Our goal during a large malaria survey of the Santa Cruz Islands in Solomon Islands was to discover how useful aurally measured temperature was to screen for those with malaria parasites. During Nov-Dec 2008, 9490 persons aged 0-75 years were screened for malaria parasites in the four island groups of Santa Cruz representing approximately half of the resident population. Thirty-seven percent claimed to have slept under a bed net the night previous to having a blood smear. A little more than 1% had fever of any description (0.91% 38-39°, 0.11% 39-40°, 0.01% >40°) while 4.4% (422/9490) overall had malaria parasites and 0.2% had gametocytes. The specificity of fever as a predictor of parasitemia as determined by blood smear was poor; 3.3% of slide positive persons had a temperature >37.5° C and 85% of those with fever were not parasitemic. Although those with fever were more likely to have malaria parasites visible on blood smear (risk rate ratio >7 for ≥38°), the vast majority (408/422; 97%) of those with parasites seen on blood smear were afebrile. Island groups varied from <1 to 11% parasite slide positive. Parasite positivity by age was 0-14y (5.3%), 15-29y (5.4%), 30-44y (4.1%), 45-59y (2.3%), >60y (0.6%). For uncertain reasons, isolated Pacific islands with endemic malaria appear to have remarkably low malaria attributable morbidity and mortality. Under the current transmission condition/setting fever although indicative of malaria, is a poor screening test which largely invalidates passive blood screening as a useful technique for malaria elimination projects. Better parasite detection methods/ algorisms are required in order to facilitate active case finding and thus parasite elimination through directed chemotherapy. .

## 197

### INFLUENCE OF ABO BLOOD GROUP PHENOTYPES ON PARITY SPECIFIC IMMUNITY TO *PLASMODIUM FALCIPARUM* MALARIA AMONG WOMEN IN PARTS OF THE IMO RIVER BASIN, NIGERIA

**Uchechukwu M. Chukwuocha**, Ikechukwu N. Dozie, Celestine O. Onwuliri, Okechukwu C. Aguwa

*Federal University of Technology, Owerri,Nigeria*

This study analyses the association between ABO blood group phenotypes in relation to placental malaria pathology and birth outcomes in the lower Imo river Basin, Nigeria. A cross-sectional study of 647 mother/child pairs delivering in Aboh Mbaise General Hospital, Aboh Mbaise Local Government Area between February-June 2007 and January-July 2008 was undertaken. Maternal peripheral and cord blood samples were obtained at delivery. Placental tissue was obtained and malaria histology classified as active, past or no malaria infection. Birth anthropometry was recorded. ABO blood group was measured by agglutination. Results showed that in primiparae, blood group O was significantly associated with increased risk of active placental infection (OR 2.18, 95% CI 1.15-4.6, p = 0.02) and an increased foetal-placental weight ratio compared to non-O phenotypes (5.68 versus 5.45, p = 0.03). In multiparae blood group O was significantly associated with less frequent active placental infection (OR 0.59, 95% CI 0.36-0.98, p = 0.04), and a higher newborn ponderal index compared to non-O phenotypes (2.65 versus 2.55, p = 0.007). In multivariate regression parity was independently associated with increased risk of placental malaria (active and past infection) in primiparae with blood group O (p = 0.034) and reduced risk in multiparae with the same phenotype (p = 0.015). Parity related susceptibility to placental malaria is associated with the mothers ABO phenotype. This interaction influences foetal and placental growth and could be an important modifying factor for pregnancy outcomes. The biological explanation could relate to sialic acid dependent placental membrane differences which vary with ABO blood group.

## 198

### ENDEMIC BURKITT LYMPHOMA IS NOT ASSOCIATED WITH COMMON SINGLE NUCLEOTIDE POLYMORPHISMS IN TOLL-LIKE RECEPTORS 4 OR 9

**David H. Mulama**

*Kenya Medical Research Institute, Kisumu, Kenya*

Burkitt lymphoma (BL) is the most prevalent pediatric cancer in equatorial Africa. Although strong epidemiological evidence links BL to early childhood infection with Epstein Barr Virus (EBV) and chronic *Plasmodium falciparum* infection, the mechanisms by which these co-infections interact to increase the risk of BL tumorigenesis are unknown. Malaria-induced pathogenesis has been implicated in disrupting immune control of EBV infection and persistence. Recent studies have linked polymorphisms in toll-like receptors (TLR) 4 and 9 with susceptibility to severe malaria. However the role of innate immunity in BL pathogenesis has not as yet been explored. In this study, Kenyan children (n=264) from regions with divergent malaria endemicity and children diagnosed with BL (n=204) were screened for the frequencies of TLR9 SNPs (T/C-1486, T/C-1174, G/A1174 and G/A2848) determined by multiplex ligation detection reaction; and TLR4 SNP (Asp299Gly) determined by real time quantitative PCR (RTQ-PCR). EBV viral load and Hemoglobin S genotype were also compared in these three groups of children stratified according to TLR polymorphism. We observed a significant association between the Kisumu control group and disease at the TLR9 T/C-1486 SNP at a probability level of 0.049. Significant association with disease was also observed in the Nandi population at the TLR9 G/A-2848 SNP at a significance level of 0.023. No other TLR9 SNPs nor the one TRL4 SNP were associated with disease at the 0.05 significance level. However, a significant difference in EBV viral load was associated with a polymorphism at the TLR9 G/A-2848 SNP at a significance level of 0.034 among the overall population and at the TLR9 G/A-1174 SNP in the control-only population. This suggests that innate immunity may play a role in determining viral set points and therefore may belong in the mechanistic pathway leading to eBL. Future functional studies are warranted to investigate the role of TLR in controlling persistent viral infections and if TLR ligands contributed by malaria co-infections modulate antiviral immunity.

## 199

### THE EFFECT OF TRANSMISSION INTENSITY AND AGE ON SUBCLASS ANTIBODY RESPONSES TO *PLASMODIUM FALCIPARUM* ANTIGENS

**Gregory S. Noland**[1], John M. Vulule[2], James W. Kazura[3], Ann M. Moormann[3], Xinan M. Min[1], Chandy C. John[1]

*[1]University of Minnesota Medical School, Minneapolis, MN, United States, [2]Kenya Medical Research Institute, Center for Global Health Research, Kisumu, Kenya, [3]Case Western Reserve University, Cleveland, OH, United States*

Adults in highland areas of unstable transmission have greater protection against clinical malaria than children in these areas, but lack the strong protection seen in adults in stable transmission areas. Differences in immunoglobulin G (IgG) subclass antibodies to *P. falciparum* antigens in these populations may play a role in the differences seen in protection against clinical malaria. IgG subclass responses to the pre-erythrocytic antigens circumsporozoite protein (CSP), liver-stage antigen 1 (LSA-1), and thrombospondin-related adhesive protein (TRAP), the pre-erythrocytic/blood-stage antigens apical membrane antigen 1 (AMA-1) and erythrocyte binding antigen-175 (EBA-175), and the blood stage antigen merozoite surface protein-1 (MSP-1) were measured by ELISA in residents of stable (n=116) and unstable (n=96) malaria transmission areas in Kenya. In the stable transmission area, >60% of residents had IgG1 antibodies and >70% of residents had IgG3 antibodies to each antigen, and these antibodies were acquired by 5 years of age. In contrast, in the unstable transmission area, frequencies of IgG1 and IgG3 responses to all six *P. falciparum* antigens continued to increase with age well into adulthood, except for MSP-1-specific IgG3 frequencies, which were similar in all age groups. IgG1 and IgG3 frequencies and levels to all antigens were significantly lower at all ages in the unstable as compared to stable transmission area, except for MSP-1-specific IgG1 and EBA-175-specific IgG3 frequencies, which were similar in both sites in individuals >15 years old, and AMA-1-specific IgG1 which was similar between sites in adults >40 years old. IgG2 responses were common in the area of stable but not unstable transmission. IgG4 responses were infrequent in both sites to all antigens except TRAP. In summary, the partial but incomplete protection against clinical malaria seen in adults in areas of unstable transmission may relate to the acquisition of IgG1 and IgG3 antibodies to blood-stage, but not pre-erythrocytic-stage, antigens.

## 200

### *PLASMODIUM VIVAX* AMA-1 PLAYS AN IMPORTANT ROLE IN ADAPTIVE IMMUNE RESPONSE ELICITING DIFFERENTIATION OF DENDRITIC CELLS

**Erika M. Braga**[1], Lilian L. Bueno[1], Ricardo T. Fujiwara[1], Cristiane G. Morais[1], Oscar Bruna-Romero[1], Erica M. Bouillet[1], Irene S. Soares[2], Cor Jesus F. Fontes[3]

*[1]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [2]Universidade de São Paulo, São Paulo, Brazil, [3]Universidade Federal de Mato Grosso, Cuiabá, Brazil*

The Apical Membrane Antigen-1 (AMA-1) is a well-characterized and functionally important merozoite protein and is currently considered a major candidate antigen for a malaria vaccine. Previously, we showed that AMA-1 has an influence on cellular immune responses of malaria-naïve subjects, resulting in an alternative activation of monocyte-derived dendritic cells and induction of a pro-inflammatory response by stimulated PBMCs. Although there is evidence, from human and animal malaria model systems, that cell mediated immunity may contribute to both protection and pathogenesis, the knowledge on cellular immune responses in vivax malaria and the factors that may regulate this immunity are poorly understood. In the current work, we describe the maturation of monocyte-derived dendritic cells of *Plasmodium vivax* naturally-infected individuals and the effect of *P. vivax* vaccine candidate Pv-AMA-1 on the immune responses of the same donors. We show that malaria-infected subjects present modulation of DC maturation, demonstrated by a significant decrease in expression of antigen-presenting molecules (CD1a, HLA-ABC and HLA-DR), accessory molecules (CD40, CD80 and CD86) and FcγRI (CD64) receptor ($P \leq 0.05$). Furthermore, Pv-AMA-1 elicits an upregulation of CD1a and HLA-DR molecules on the surface of monocyte-derived dendritic cells ($P = 0.0356$ and $P = 0.0196$, respectively), and it is presented by AMA-1-stimulated DCs. A significant pro-inflammatory response elicited by Pv-AMA-1-pulsed PBMCs is also demonstrated, as determined by significant production of TNF-α, IL-12p40 and IFN-γ ($P \leq 0.05$). Our results suggest that Pv-AMA-1 may partially revert DC down-modulation observed in infected subjects, and exert an important role in the initiation of pro-inflammatory immune responses that might contribute substantially to protection.

## 201

### ERYTHROCYTE CR1/CD35 INHIBITS TNF-A PRODUCTION BY RESTRICTING IMMUNE COMPLEX UPTAKE BY MACROPHAGES

**Michael M. Odera**[1], Jose A. Stoute[2]

*[1]Kenya Medical Research Institute, Kisumu, Kenya, [2]Department of Medicine, Penn State University College of Medicine, Hershey, PA, United States*

Individuals suffering from severe malaria have elevated concentrations of TNF-α, increased levels of circulating immune complexes (ICs) and decreased levels of CR1/CD35 on their erythrocytes. We postulated that erythrocytes can serve a dual role during malaria infection. Erythrocytes bind C3b opsonized ICs via CR1/CD35 and restrict stimulation of

macrophages. However, under some circumstances such as during slow circulation in sequestered capillaries, IC-loaded erythrocytes can stimulate macrophages to produce pro-inflammatory cytokines such as TNF-α. Using flow cytometry, we identified individuals who were low, medium, and high CR1 expressors from a cross-sectional survey that was recently carried out. 45 individuals were selected (15 individuals per CR1 category) and IC binding capacity determined by flow cytometry. Using an *in vitro* system, macrophages were stimulated with opsonized BSA-anti-BSA ICs, a cocktail of opsonized ICs and erythrocytes, IC-loaded erythrocytes, plain erythrocytes, IgG, or LPS. Supernatants were harvested after 8 hours of incubation and TNF-α was measured by ELISA. Erythrocytes inhibited IC-induced TNF-α production by macrophages and in a manner proportional to the CR1 levels. Also, IC-loaded erythrocytes stimulated macrophages to release TNF-α. We conclude that erythrocyte CR1/CD35 may act as a dynamic buffering system that can prevent ICs from stimulating macrophages to release TNF-α. However, CR1/CD35 enables erythrocytes to present ICs to macrophages leading to stimulation and secretion of TNF-α.

## 202

### EXPRESSION OF FCγRIII BY MONOCYTES FROM CHILDREN WITH *PLASMODIUM FALCIPARUM* MALARIA: ASSOCIATION WITH SEVERE MALARIAL ANAEMIA

**Lilian A. Ogonda**[1], Alloys S. Orago[2], Michael F. Otieno[2], Jose A. Stoute[3]

*[1]Kenya Medical Research Institute (KEMRI)/Walter Reed Project (WRP), Kisumu, Kenya, [2]Kenyatta University, Nairobi, Kenya, [3]Pennsylvania State University College of Medicine, Hersey, PA, United States*

Macrophages play an important role in the innate immune response to malaria. Malaria infection leads to formation of immune complexes (ICs) that can interact with monocyte/macrophages by binding to their surface Fc γ receptors. Fc γ receptor IIIa (FcγRIIIa, CD16a) expressed on monocytes/macrophages can be cross-linked by immune complexes resulting in production of tumor necrosis factor alpha (TNF-α), a cytokine implicated in the development of severe malaria. FcγRIIIa can also mediate phagocytosis of antibody-coated infected and uninfected red cells which could contribute to the development of severe anemia. Therefore, expression levels of FcγRIIIa may influence an individuals' susceptibility to severe *Plasmodium falciparum* malaria. We investigated the expression of FcγRIIIa on monocytes of children with severe malarial anaemia, cerebral malaria and their age and gender-matched uncomplicated malaria controls by flow cytometry. Whole blood was obtained from the patients during the acute illness and after recovery from illness and stained with directly conjugated antibodies against CD14 and CD16a/FcγRIIIa followed by red cell lysis. In addition, we stimulated monocytes with BSA-anti-BSA immune complexes to determine the effect on the intracellular expression of TNF-α by monocytes. The expression of FcγRIIIa on monocytes was highest in children with severe malarial anaemia compared to all the other groups at enrollment. Furthermore, there was significant inverse correlation between haematocrit levels and FcγRIIIa expression levels on monocytes. The intracellular TNF-α expression by CD14+CD16+ monocyte sub-population in response to immune complex stimulation correlated positively with their FcγRIIIa expression. These data suggest that children who are predisposed to severe malarial anaemia may overexpress FcγRIIIa during malaria infection which may lead to FcγRIIIa-mediated red cell destruction via erythrophagocytosis and increased TNF-α production.

## 203

### IFN-Γ RESPONSES TO *PLASMODIUM FALCIPARUM* ANTIGENS IN AREAS OF UNSTABLE TRANSMISSION DECREASE IN PERIODS OF VERY LOW OR ABSENT TRANSMISSION

**Bilha S. Ogola**[1], Gregory S. Noland[2], Ng'wena G. Magak[3], Cyrus Ayieko[4], John M. Vulule[5], Chandy C. John[2]

*[1]Moi University, Department of Biological Sciences, School of Science, Eldoret, Kenya, [2]University of Minnesota Medical School, Minneapolis, MN, United States, [3]Moi University, School of Medicine, Eldoret, Kenya, [4]Maseno University, Department of Zoology, Faculty of Science, Maseno,Kenya, [5]Kenya Medical Research Institute, Center for Global Health Research, Kisumu, Kenya*

Immune responses to *Plasmodium falciparum* in areas of unstable malaria transmission, such as highland areas of Western Kenya, differ considerably from those in stable transmission areas. The longevity of cellular immunity in the absence of sustained transmission in these areas has not been described. Interferon γ (IFN-γ) cytokine responses to pre-erythrocytic (CSP, LSA-1, and TRAP), blood-stage (MSP-1) and multi-stage (AMA-1 and MB2), vaccine candidate antigens were measured by ELISA in PBMC culture supernatants from individuals residing in two unstable transmission areas of higher (n=151) and lower (n=141) baseline malaria incidence. Samples from the same individuals were collected in April and October 2008. During this period, cross sectional surveys showed an absence of asymptomatic parasitemia in the area and no microscopy positive cases of clinical malaria were seen in the area. Frequency of IFN-γ responses to all malaria antigens, except TRAP, declined by >2-fold between April (range 8.9-39.0%) and October 2008 (range 2.4-18.5%). Geometric mean levels of IFN-γ to CSP, LSA-1, MB2, and MSP-1 were also significantly reduced during this period. While there was no significant difference in frequency or levels of responses between sites in April, frequency of MB2 responses and geometric mean levels of MB2, LSA-1, and MSP-1 were significantly lower during October in the site with lower baseline transmission. IFN-γ responses to *P. falciparum* antigens in areas of unstable transmission wane during prolonged periods of low or absent transmission and wane more quickly in areas of historically less intense transmission.

## 204

### CYTOKINE RESPONSE TO *PLASMODIUM FALCIPARUM* MULTISTAGE ANTIGEN (MB2) IN ADULTS LIVING IN AREAS OF VARYING MALARIA TRANSMISSION IN WESTERN KENYA

**Gideon M. Ng'wena**[1], Lyticia V. Ochola[2], Gregory S. Noland[3], Anthony A. James[4], Ayub V. Ofulla[2], Vulule M. John[5], Chandy C. John[3]

*[1]Moi University School Of Medicine, Eldoret, Kenya, [2]Maseno University, Kisumu, Kenya, [3]Minnesota Medical School, Minneapolis, MN, United States, [4]Department of Molecular Biochemistry, Irvine, CA, United States, [5]Kenya Medical Research Institute, Kisumu, Kenya*

Malaria immunity in areas of stable, perennial malaria transmission differs considerably from that in epidemic-prone areas with unstable transmission. *Plasmodium falciparum* MB2 antigen is a novel protein expressed in various stages of the parasite's life cycle and could be exploited as a vaccine candidate. We evaluated the interferon γ (IFN-γ) and interleukin-10 (IL-10) responses in PBMC culture supernatants to MB2 by ELISA and ELISPOT in 177 adults residing in Kipsamoite, a highland site in western Kenya with unstable malaria transmission, and Kanyawegi, a lowland site with stable malaria transmission. Frequencies of individuals with a positive IFN-γ response to MB2 by ELISA were similar in the unstable (27.5%) and stable-transmission areas (18.8%; *P*=0.33). Geometric mean levels of IFN-γ were also similar between unstable 17.57 pg/ml (range 0-1064.22 pg/ml) and stable 113.91 pg/ml (range 0-2896 pg/ml) transmission areas (*P*=0.26). Likewise, MB2-specific IL-10 frequencies (22.5% vs. 25%; *P*=0.78) and mean levels (39.56 pg/ml [range 0-353.4 pg/ml] vs. 65.31 pg/ml [range 0-506.9 pg/ml]; *P*=0.55) were similar between unstable and stable transmission areas. Frequencies of IFN-γ

secreting cells measured by ELISPOT were similar in both areas (12.5% and 12.3%, respectively, *P*=0.97). These results indicate that MB2 induces significant antigen specific cytokine responses that do not differ with malaria transmission intensity. Further studies are required to investigate whether IFN-γ or IL-10 responses to MB2 correlate with protection from infection or disease.

## 205

### HUMAN BLOOD MONOCYTE PHENOTYPE AND FUNCTION DURING ACUTE UNCOMPLICATED MALARIA ATTACK

**Pattamawan Chimma**[1], Christian Roussilhon[1], Panudda Sratongno[2], Kovit Pattanapanyasat[2], Jean-Louis Pérignon[1], David Roberts[3], Pierre Druilhe[1]

*[1]Institut Pasteur, Paris, France, [2]Mahidol University, Bangkok, Thailand, [3]Oxford University, Oxford, United Kingdom*

The relative contribution of MO to either protection or pathogenesis was characterized in patients with acute uncomplicated malaria attacks enrolled in Thailand. We compared the phenotypes of blood MO from healthy malaria exposed individuals to that of MO obtained from patients with acute, uncomplicated episodes of *Plasmodium falciparum* infection. In addition, the anti-parasitic activities of the blood MO originating from either healthy individuals or from patients were evaluated by phagocytosis and ADCI tests. Compared to the circulating MO of malaria naïve donors, the blood MO of malaria exposed but healthy individuals expressed high levels of maturation markers, and at least 98% of cells expressed HLA-DR and TREM-1, two activation markers. This demonstrates that MO of healthy donors living in endemic areas differ from MO of healthy malaria naïve donors. In patients, both a dramatic increase in the number of CD16+ MO and raised Fluorescence intensity of TREM-1 expression were detected. Two groups of patients with the same clinical parameters and barely detectable levels of anti-malaria antibody levels were delineated on the exclusive basis of CCR2+/CX3CR1+/CD14high expression. The use of the same pool of purified immune IgG in all the functional assays contributed to highlight the existence of some level of heterogeneity both in the phenotype and the functional capacity of the blood MO. This indicates that so far undetected differences in the apparently homogeneous clinical presentation of uncomplicated malaria attacks. Therefore, these original and stimulating new results contribute to shed light on major events regarding the complex early relationships between blood MO from patients with uncomplicated malaria and *P. falciparum* parasites. These observations also underscore the fundamental contribution of innate immunity in the early control of falciparum parasitaemia.

## 206

### HOST GENOTYPE FOR DUFFY ANTIGEN RECEPTOR CHEMOKINE (DARC) MODIFIES ACQUISITION OF IMMUNE RESPONSE TO *PLASMODIUM VIVAX*

**Amanda Maestre**[1], Carlos Muskus[2], Victoria Duque[2], Olga Agudelo[1], Akihide Takagi[3], Kim Lee Sim[4], Stephen L. Hoffman[5], Sope Olugbile[3], Ivan D. Velez[2], Ruobing Wang[3]

*[1]Grupo Salud y Comunidad, Universidad de Antioquia, Medellin, Colombia, [2]Programa de Estudio y Control de Enfermedades Tropicales PECET, Universidad de Antioquia, Medellin, Colombia, [3]Seattle Biomedical Research Institute, Seattle, WA, United States, [4]Protein Potential, Rockville, MD, United States, [6]Sanaria, Rockville, MD, United States*

Individuals that lack Duffy Antigen Receptors for Chemokines (DARC) on their red blood cells (Duffy negative) are 'resistant' to vivax blood stage infection as *Plasmodium vivax* parasite requires interaction with DARC for its invasion. The level of expression of duffy antigen which varies between different DARC genotypes (Duffy positive) is also associated with the level of susceptibility to vivax infection. Within a population that resides in malaria-endemic regions of Colombia, we observed that the level of naturally-induced antibodies against vivax blood stage antigen (PvMSP1) varies with DARC genotype, thus with the level of DARC expression. Duffy positive individuals were more likely to have anti-PvMSP1 antibodies than those that are duffy negative. Interestingly also, those that had one negative allele (FY*B/FY*Bnull and FY*A/FY*Bnull) were more likely to have anti-PvMSP1 antibodies when compared to those with double positive allele (FY*B/FY*B and FY*A/FY*B, respectively). They were also more likely to have antibodies against vivax liver stage antigen (PvCSP). This may be due to the fact that the higher susceptibility to infection observed in individuals with double positive alleles also generate greater suppression of immune response that malaria antigens are known to induce. No association between DARC expression and the presence of antibodies specific for liver and blood stage falciparum antigens (PfCSP and PfMSP1) was observed as they do not require interaction with DARC.

## 207

### EPSTEIN BARR VIRAL LOAD AND ANTIBODIES IN CHILDREN WITH MALARIA

**Moses Y. Otiende**

*Walter Reed Project/United States Army Medical Research Unit-Kenya (USAMRU-K), Kisumu, Kenya*

Epstein Barr virus (EBV) and *Plasmodium falciparum* malaria have an overlapping distribution and are incriminated as the primary causal agents for endemic Burkitt's lymphoma. In this study we determined the viral load as well as the serological signatures to various EBV antigens in children with complicated and uncomplicated malaria. Children with severe malarial anemia (SMA) were age and sex matched to those with uncomplicated malaria (UM). EBV-DNA viral load was quantified by real time quantitative PCR and viral load extrapolated from a standard graph from a sample with known copy numbers. IgG and IgM antibody levels to viral capsid antigen (VCA) and EBV nuclear antigen (EBNA) were determined by ELISA. B-cell numbers that are target for EBV were enumerated by flow cytometer. Of the 94 subjects analyzed, 53% (N=25 in each arm) had detectable EBV load. The geometric mean viral copy number/mL was higher in SMA (27,556± 25,377 SE) compared to UM (21,703±17,434 SE), although the difference was not statistically significant. The mean B-cell numbers were higher in cases (1561±467 SE) than controls (1246±278 SE). The geometric mean VCA IgG titers were higher in the cases (0.97± 0.05 SE) compared to controls (0.79± 0.06 SE ± P≤0.04). There was no difference in the titers of the other antibodies analyzed. In conclusion, our findings are consistent with early childhood exposure to EBV. We attribute the higher viral load and VCA IgG in the SMA to the expansion of infected B-lymphocytes, probably fueled by malaria antigenic stimuli.

## 208

### THE EFFECTIVENESS OF SULFADOXINE - PYRIMETHAMINE-BASED COMBINATION THERAPY VERSUS SULFADOXINE - PYRIMETHAMINE ALONE AGAINST UNCOMPLICATED *PLASMODIUM FALCIPARUM* INFECTIONS

**Zoumana I. Traore**, Cheick Papa Sangare, Abdoul Habib Beavogui Ah, Hamma Maiga, Mamadou Tekete, Dinkorma Ouologuem, Antoine Dara, Christelle N'Dong, Oumar Bila Traore, Abdoulaye Djimde, Ogobara K. Doumbo

*Malaria Research and Training Center, Bamako, Mali*

*Plasmodium falciparum* drug resistance has emerged as a critical public health problem in many parts of the world, including Mali. Resistance to sulfadoxine-pyrimethamine (SP) in particular has been widely reported and is spreading. To improve efficacy and limit the spread of drug resistance, SP-based combination therapies which include artemisinins have been advocated recently, but artemisinins are expensive and relatively untested in highly endemic areas. In the present study, we tested the effectiveness of SP-based combination therapies versus SP alone. From 2004-2005, we carried out an Open-Label Randomized Trial of the effectiveness of sulfadoxine-pyrimethamine + artesunate (SP+AS), sulfadoxine-

pyrimethamine + amodiaquine (SP+AQ), and sulfadoxine-pyrimethamine (SP) alone, for the treatment of uncomplicated malaria in two Malian savannah villages: Kollé and Bancoumana. The efficacies of these therapies was assessed in the treatment of uncomplicated malaria among 736 children under five years (with a rate of 6% of lost sight) in Bancoumana and Kollé using the WHO 2003 *in vivo* test standard of 28 days. These children was randomized between SP+AQ (n=244), SP+AS (n=251) and SP alone (n=241), and treatment outcomes after 28 days were determined. Total treatment failure of 0.4% was observed for SP+AS, 1,6% for SP Alone, and no treatment failure was observed for SP+AQ. The peak of porting gametocytes carriage was observed in all treatment regimens: SP=32.3%, SP + AQ = 18.3% and SP + AR = 16.5%. We used the polymerase chain reaction to assess the rate of punctual mutations conferring the resistance of *P. falciparum* to SP at Day 0 and the results showed: pfdhfr 38,2%, 37,3% and 43,1% respectively for the 51, 59 and 108 codons, for pfdhps 14, 4% 437 codon. No mutations were observed on pfdhps 540 codon. Polymorphism analyses by PCR showed 19.4% for MSP1 (n=91), 41.5 for MSP2 (n=94) and 12.4 for the Ca1 microsatellite (n=97). In summary, we observed that SP-based combination therapies were more efficacious compared to SP alone. and SP+AQ was the most efficacious combination. Therefore, SP should be used in combination with other artemisin antimalarials for the treatment of uncomplicated malaria.

## 209

### TRANSGENIC MODEL FOR ANTI-*PLASMODIUM VIVAX* DIHYDROFOLATE REDUCTASE-THYMIDYLATE SYNTHASE SCREENING

**Voravuth Somsak**[1], Chairat Uthaipibull[2], S. Srichairatanakool[1], Y. Yuthavong[2], S. Kamchonwongpaisan[2]

*Chiang Mai University, Chiang Mai, Thailand, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Pathumthani, Thailand*

Discovery of drug against *Plasmodium vivax* is hampered by the lack of routine continuous culture in the laboratory of this parasite. Yeast and bacterial surrogates carrying *P. vivax* dihydrofolate reductase-thymidylate synthase (PvDHFR-TS), which complements chemically knocked out host DHFR, were used as antifolate screening models. Here, we generated transgenic rodent malaria parasite *P. berghei* expressing PvDHFR-TS as an *in vivo* model for antifolate screening. In order to generate *P. berghei* expressing PvDHFR-TS, endogenous *dhfr-ts* gene of *P. berghei* was replaced with either wild-type or double mutant (S58R/S117N; SP21) of *P. vivax dhfr-ts* genes via double cross-over homologous recombination. Targeted gene replacement was confirmed by PCR with specific primers and southern blot analysis of digested parasite genomic DNA. Transgenic *P. berghei* expressing PvDHFR-TS displayed a normal blood-stage growth rate when compared with wild type *P. berghei*. For *in vivo* model validation, pyrimethamine and chloroquine were used in a standard 4-day suppressive test. The ED50 of pyrimethamine (Pyr) against wild type *P. berghei* and wild type PvDHFR-TS-dependent transgenic parasite was 0.5 mg/kg, whereas ED50 of Pyr against SP21 transgenic parasite was 30 mg/kg. The SP21 transgenic parasite was 60 folds more resistant than wild type *P. berghei* and wild type PvDHFR-TS transgenic parasites. In addition, for chloroquine treatment, the ED50 values of all parasites were 2 mg/kg, the same as chloroquine sensitive strain of *P. berghei*. These *in vivo* antifolate screening models against PvDHFR-TS are useful for development of new antifolate compounds, for which a routine continuous culture of *P. vivax* parasite is not yet available.

## 210

### STUDIES ON ABO BLOOD GROUPS, HAEMOGLOBINOPATHIES AND G6PD GENOTYPES, AND *PLASMODIUM FALCIPARUM* INFECTION IN KPONE-ON-SEA, GHANA

**Samuel Armoo**

*Noguchi Memorial Institute for Medical Research, Legon, Accra, Ghana*

Erythrocyte variants such as the ABO blood groups, haemoglobinopathies and G6PD genotypes are known to be associated with naturally acquired immunity against malaria. Despite evidence of their protection, some epidemiological studies have proved otherwise. Therefore their associations with malaria at Kpone-On-Sea, a coastal fishing village with high malaria incidence, was determined. A cross-sectional study of 592 randomly selected individuals. Human DNA was extracted using DNeasy Kit (Qiagen, USA). To determine the blood groups and haemoglobinopathies the standard agglutination method and cellulose acetate haemoglobin electrophoresis were used respectively. G6PD genotypes were determined by a PCR-based method using primers NA4: 5'-CCTGTTCCCTCTGCCACA-3' and NB4: 5'-GGGGGTCTCAAGAAGTAC-3'. Then followed by restriction with Hsp 92II enzyme. Parasitaemia was determined by microscopy. Statistical methods used include ANOVA, T-tests and chi-square tests. In all, 44.76%, 20.61%, 31.25% and 3.38% had blood group O, A, B and AB respectively. The prevalences of HbAA, HbAC, HbAF, HbAS, HbSC and HbSS were 71.28%, 8.11%, 1.18%, 16.89%, 1.35% and 1.01% respectively. Also 50.68%, 35.81%, 8.11%, 1.69% and 3.72% were G6PD homozygous normal, hemizygous normal, heterozygous deficient, homozygous deficient and hemizygous deficient respectively. Only 72 individuals were parasitaemic. Blood group O was not associated with reduced parasitaemia (P = 0.587). HbAS and HbAC were not associated with reduced parasitaemia, (P = 0.212) and (P = 0.851) respectively. The heterozygous G6PD deficiency was not associated with reduced parasitaemia (P = 0.664). Sampling occurred in a period following a long dry season, resulting in recording of low parasitaemia levels which may have affected the prevailing association between the RBC variants and parasitaemia. A more sensitive diagnostic technique such as PCR should be used in future studies to determine parasitaemia. There may be a clinal trend in the distribution of HbS and HbC in the country so the need for nationwide screening.

## 211

### RESISTANCE-MEDIATING POLYMORPHISMS OF *PLASMODIUM FALCIPARUM* AMONG SEVERE MALARIA PATIENTS PRESENTING TO THE KOMFO ANOKYE TEACHING HOSPITAL, KUMASI, GHANA

**Harry H. Abruquah**[1], Jennifer Evans[2], Daniella Tominsky[3], Christa Flessner[3], Florian Marks[3], Juergen May[3], Sammy C. Tay[4], Edward T. Agbenyega[4], Rolf Hotsmann[3]

*[1]University Hospital, Kumasi, Ghana, [2]KCCR, Kumasi, Ghana, [3]BNI, Hamburg, Germany, [4]School of Medical Sciences, Kumasi, Ghana*

While shortages of resources undeniably pose significant obstacles to malaria control in many developing countries, drug resistance has been a major additional contributor to the failure of the battle against malaria in many of these countries. Of 140 children presenting with severe malaria to the Komfo Anokye Teaching Hospital between November 2000 and February 2001, 109 (77.9%) had detectable pre-treatment chloroquine levels. A molecular study was conducted to determine the presence of mutant alleles of the two genes associated with chloroquine resistance among the isolates of *Plasmodium falciparum* from these children. Polymerase Chain Reaction and restriction digestion analysis of *P. falciparum* chloroquine resistant transporter gene (*Pfcrt*) indicated that 123 (87.9%) had the mutant T76 gene, and that *Pfcrt* mutant T76 gene correlated well with higher chloroquine levels (P >0.01). Sequencing analysis of these showed consistent genetic sequences for chloroquine

resistant and sensitive parasites with respect to *Pfcrt* amino acid positions 72, 73, 74, 75, and 76 (P >0.01), but inconsistent genetic sequences with respect to *Pfmdr*-1 amino acid positions 86 and 184; all samples were mutated at amino acid positions 1034, 1042, and 1246 of the *Pfmdr*-1 gene. The *Pfcrt* T76 mutation was found in 88.4% of the samples having the *P. falciparum* multi-drug resistance 1 (*Pfmdr*-1) Y86 mutation (odds ratio (OR) =4.8 [95% CI: 1.7-13.3]; P=0.002). The *Pfmdr*-1 Y86 mutation was found in 67.6% of the samples having the *Pfcrt* T76 mutation. This suggests that both mutations, occurring on two different chromosomes, are independently selected by plasma chloroquine levels and that one mutation (Y86) might modify/increase the effect of the other (T76). These results are consistent with other studies, and affirm *Pfcrt* as a better chloroquine resistant marker over *Pfmdr*-1. The data show that a high proportion of children admitted at the Komfo Anokye Teaching Hospital had sub-therapeutic pretreatment chloroquine levels. This study also confirms the true picture of the much-overlooked antimalarial drug resistance situation in the area and recognizes the need for a proper treatment strategy.

## 212

### REGULATORY MECHANISMS OF GENE EXPRESSION IN INTRAERYTHROCYTIC CELL CYCLE OF *PLASMODIUM FALCIPARUM*

**Kanako Komaki-Yasuda**[1], Mitsuru Okuwaki[2], Kyosuke Nagata[2], Shin-ichiro Kawazu[3], Shigeyuki Kano[1]

*[1]Research Institute, International Medical Center of Japan, Tokyo, Japan, [2]Graduate School of Comprehensive Human Sciences and Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba, Japan, [3]National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan*

*Plasmodium falciparum*, the human malaria parasite, is evolutionarily distant from other eukaryotes. Genome-wide analyses of transcription-associated proteins have revealed a relative paucity of putative regulatory transcription factors and an abundance of putative chromatin remodeling machinery, suggesting that this parasite has a transcription regulatory system that is distinct from those of other eukaryotes. As model cases for stage specific transcription in *P. falciparum* erythrocytic stage, we have analyzed transcriptional regulation of the peroxiredoxin genes, *pf1-cys-prx* and *pftpx*-1, which show trophozoite/schizont stage specific and constitutive expression patterns respectively. The reporter assays revealed the presence of putative enhancers in the 5′ regions of these genes. Although *pf1-cys-prx* shows trophozoite/schizont stage-specific transcription, a novel putative *cis*-acting enhancer sequence in *pf1-cys-prx* was constitutively active when inserted into the 5′ region of *pftpx-1*. These findings indicated that the chromatin structure of endogenous *pf1-cys-prx* might contribute to the stage-specific expression. Electrophoretic mobility shift and DNase I footprinting assays showed that the *pf1-cys-prx* enhancer is the target of trophozoite/schizont stage-specific DNA binding nuclear proteins. In addition, chromatin immunoprecipitation assays showed that the increased levels of histone acetylation in the 5′ region of *pf1-cys-prx* and *pftpx-1* correlated with the transcriptional activity of these genes. Recruitment of PfGCN5 histone acetyltransferase to the *pf1-cys-prx* enhancer in trophozoite/schizont stage was observed. These results suggest that *P. falciparum* possesses a sophisticated system of transcriptional regulation during intraerythrocytic stages that is managed by coordinated interactions of unique *cis*-elements and *trans*-acting factors and chromatin modifications. The nuclear factor which associates specifically with *cis*-acting enhancer region of *pf1-cys-prx* is currently under its purification and identification.

## 213

### ANALYSIS OF PFE0565W AND PF11_0394, TWO *PLASMODIUM FALCIPARUM* GENES

**Maggie Schlarman**[1], Michael Kariuki[1], Alexis LaCrue[2], Renee Roberts[1], Ruguang Ou[1], Brenda Beerntsen[1]

*[1]University of Missouri, Columbia, MO, United States, [2]University of South Florida, Tampa, FL, United States*

Malaria is a resurging disease due, in part, to resistance that has developed in *Plasmodium spp.* and mosquitoes against drugs and insecticides, respectively. Because of this disease resurgence, better control methods are in great need. A key stage in the *Plasmodium* life cycle is the sporozoite because it exhibits dual infectivity in both the mosquito vector and vertebrate host and, therefore, is a promising target for discovering effective ways of controlling malaria. The *P. falciparum* genes, PFE0565w and PF11_0394, were chosen as candidates for study due to their potential role in the invasion of host tissues. These genes were selected based on data from PlasmoDB, indicating that these genes are expressed both at the transcriptional and protein levels in sporozoites and likely encode putative surface proteins. Additional sequence analysis shows that each gene has orthologs in other *Plasmodium* species and PF11_0394 also has orthologs in other Apicomplexans. Both PFE0565w and PF11_0394 express transcripts during both the sporozoite and erythrocytic stages of the parasite life cycle. PFE0565w protein appears to be expressed during the asexual stages of the life cycle, as suggested by Western blot analysis and confocal microscopy, and shown to be expressed on the sporozoite surface by confocal microscopy. PF11_0394 protein expression studies are currently in progress using antibodies generated against PF11_0394 peptides. Also, GFP trafficking constructs have been made to track the expression of both the PFE0565w and PF11_0394 proteins throughout the parasite's life cycle and to confirm protein expression results mentioned above. Furthermore, both gene disruption and knock-out constructs have been successfully created for both genes and studies are in progress to assess the function of the genes in parasite development and to determine if they play a role in host tissue invasion. Lastly, a comparative study between the *P. berghei* orthologs of PFE0565w and PF11_0394, PB107985.00.0 and PB000481.01.0, respectively, is in progress.

## 214

### FUNCTIONAL CHARACTERIZATION OF *PLASMODIUM OVALE* DIHYDROFOLATE REDUCTASE-THYMIDYLATE SYNTHASE

**Srisuda Tirakarn**[1], Ubolsree Leartsakulpanich[2], Palangpon Kongsaeree[1], Yongyuth Yuthawong[2]

*[1]Department of Chemistry and Center for Excellence in Protein Structure and Function, Faculty of Science, Mahidol University, Bangkok, Thailand, [2]National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Thailand Science Park, Paholyothin Rd, Pathumthani, Thailand*

*Plasmodium ovale* is one of the four human malaria parasites distributed mostly in Sub-Saharan Africa, New Guinea and Southeast Asia. Co-infection of *P. ovale* with other malaria species is usually reported. Its ability to relapse and difficulty to differentiate from others by blood smear method underestimate the actual burden caused by this parasite. Although the emergence of drug resistance is widely known for *P. falciparum* and *P. vivax*, the problem has never been addressed for *P. ovale*. Recently, a putative gene encoding PoDihydrofolate reductase-thymidylate synthase (DHFR-TS), a bifunctional enzyme targeted by antifols, was sequenced. In order to establish the function of the putative PoDHFR-TS, the gene was cloned, expressed, and characterized for kinetic properties including the antifol sensitivity. Complementation assays revealed that transformed PoDHFR-TS could functionally complement both *Escherichia coli* DHFR under trimethoprim treatment and TS-deficient *E. coli* stain. PoDHFR-TS was overexpressed as a soluble protein, which was purified by Methotrexate-affinity chromatography. In addition to the 74 kDa full length PoDHFR-TS, proteins with molecular weights of 35 kDa and 32 kDa

were obtained. Results from N-terminal sequencing and LC-MS indicate that they are PoDHFR-TS. Incomplete sequences may arise from protein degradation or incomplete translation as postulated for the expression of PfDHFR-TS. The partial purified protein exhibited DHFR activity as determined by monitoring the consumption of NADPH to reduce DHF to THF. The enzyme was competitive inhibited by pyrimethamine and cycloguanil with the sensitivity at nano-molar level, comparable to that reported for the wildtype DHFR-TS from other *Plamodium* species. Our study demonstrated the DHFR-TS function for putative gene and denoted the antifol susceptibility of PoDHFR-TS. The results suggest that chemotherapy with the combination of antifol can be deployed in Po coinfected endemic area.

## 215

### FUNCTIONAL CHARACTERISATION OF THE *PLASMODIUM* CENTROMERE AND GENERATION OF A *PLASMODIUM* ARTIFICIAL CHROMOSOME

**Shiroh Iwanaga**[1], Masao Yuda[1], Izumi Kaneko[1], Chris J. Janse[2], Andrew P. Waters[3]

*[1]Mie University, Tsu, Japan, [2]Leiden University Medical Center, Leiden, The Netherlands, [3]University of Glasgow, Glasgow, United Kingdom*

Centromeres of the protozoan malaria parasite, *Plasmodium falciparum*, have been identified within 2-3kb ultra-high AT-rich DNA, gene-barren, chromosomal regions that are syntenically conserved in chromosomes with other Plasmodium species. In this study a comparative analysis of several putative centromeres (PCEN) from three Plasmodium species reveals that, while highly divergent in sequence, centromeres consist of a general core AT-rich (CAT) region flanked by either one or two repetitive elements. The addition of PCEN elements to plasmids in transfection experiments on blood-stage P. berghei parasites clearly demonstrated the efficient segregation and maintenance of episomal plasmid DNA during mitosis, in the absence of drug pressure. Efficient segregation of plasmids requires both the CAT and repetitive regions. PCEN-containing plasmids are also stably maintained during meiosis and mitosis throughout the mosquito stages but unexpectedly segregation is less efficient during cell division in the liver. However, the addition of telomere sequences to PCEN containing DNA plasmids increased the efficiency of segregation of these DNA constructs to the same level observed in blood-stages. Efficient segregation and maintenance was also observed when parasites were transfected with linear constructs containing both PCEN elements and telomeres. We not only report the first functional analysis of Plasmodium centromeres but also the generation of a Plasmodium Artificial Chromosome (PAC), which may help shed light on the parasites genome biology and expand the so-far limited set of genetic tools for transformation of Plasmodium.

## 216

### LOCALIZATION OF SERINE HYDROXYMETHYLTRANSFERASE ISOFORMS IN *PLASMODIUM FALCIPARUM*

**Wichai Pornthanakasem**, Ubolsree Leartsakulpanich, Chairat Uthaipibull, Darin Kongkasuriyachai, Yongyuth Yuthavong

*National Center for Genetic Engineering and Biotechnology, Pathumthani, Thailand*

Serine hydroxymethyltransferase (SHMT), a pyridoxal phosphate-dependent enzyme in the dTMP cycle, converts serine and tetrahydrofolate to glycine and methylene tetrahydrofolate. Since *Plasmodium* malaria parasite relies solely on *de novo* pyrimidine biosynthesis pathway which differs from human, SHMT has been proposed to be a potential antimalarial drug target. Based on the DNA sequence search from PlasmoDB, there are two genes encoding SHMT in *P. falciparum*; one of which has a putative transit peptide at N-terminus. The presence of two isozymes in *P. falciparum* that may implicate the drug development is yet to be established. Along with other studies on gene disruption and enzyme characterization to validate these proteins as the drug targets, the localization study of these isoforms is investigated. The putative cytosolic and mitochondria SHMT genes were cloned in frame with DsRed and green fluorescence protein respectively. The cytosolic SHMT construct contains human DHFR, whereas the mitochondrial SHMT construct contains BSD as a selectable marker. These plasmids were successfully transfected into *P. falciparum*. Using transfection technique, SHMT-fusion proteins can be expressed. Preliminary results showed different distribution patterns of SHMT-fusion proteins in transfected parasites. Specific organelle localization will be confirmed by other methods. This study provides additional support evidence that *P. falciparum* has two forms of SHMT.

## 217

### KNOWLEDGE, ATTITUDES AND PRACTICES OF MOTHERS ON THE USE OF INSECTICIDE-TREATED NETS IN THE PREVENTION OF CHILDHOOD MALARIA IN A CHILD-IMMUNIZATION CLINIC IN SOUTH-EASTERN NIGERIA

**Kenechukwu O. Chudy-Onwugaje**

*Nnamdi Azikiwe University, Nnewi, Anambra State, Nigeria*

Malaria is a major cause of morbidity and mortality in the non-immune under-five population of Africa. The renewed drive towards the use of insecticide treated nets (ITNs) as a form of vector-control is crucial in the prevention of malaria in this population. However, the target of the African Summit on Roll back malaria to provide protection with ITNs for at least 60% of children and pregnant women by 2005 has not been met even in year 2009.This study was done to assess the perceptions and practices of mothers attending a child-immunization clinic in South-Eastern Nigeria on the use of ITNs and to identify ways of improving their use. Semi-structured questionnaires were administered to 112 mothers who had brought their children to the child-immunization clinic of Nnamdi Azikiwe University Teaching Hospital, a tertiary hospital in Nnewi, Nigeria. There was good knowledge on malaria transmission and the use of ITNs in its prevention with 59(53%) of the mothers citing the ante-natal clinics as their main source of information. 49(44%) of the respondents had at least one ITN in their household. 56(89%) of the respondents who did not own ITNs expressed a desire to own and use them. However, only 25(51%) of those that owned ITNs had used them to protect their infants and other children in the week before their attendance to the clinic. Cost of purchase, cost of retreatment and local availability were the major reasons why some respondents did not own insecticide-treated nets. Resources need to be focused on programmes that encourage daily use of ITNs in households. The ante-natal clinics seem to be a veritable vehicle for this as a majority of the women identified them as their main source of information. Public-private partnerships are needed in the provision of subsidized nets and in the retreatment of these nets as cost and availability are major obstacles to ownership.

## 218

### ANTIMALARIAL MEDIATED MODULATION F MELANIZATION AND INVOLVEMENT OF PROPHENOLOXIDASE IN THE SUPPRESSION OF *PLASMODIUM* DEVELOPMENT IN *ANOPHELES*

**Fusheng Huang**, Jian Zhang, Ying Wang

*Third Military Medical University, Chongqing, China*

It is intriguing to note that the patterns of melanotic response are different in these two vector mosquitoes. Nitroquine was an effective antimalarial drug. In order to explore the effect of nitroquine on mosquito immunity, we detected the activity increase of phenoloxidase (PO) enzyme, with the nitroquine treatment. Moreover, we cloned prophenoloxidase (PPO ) genes which is accepted as the inactive PO form and observed inducible expression of this gene with nitroquine treatment by real-time PCR. Our data implied that up-regulation of PPO gene and PO activity might be correlated with the nitroquine. Hence, we supposed the involvement of *An.stephensi* TEP1 in melanization induced by nitroquine. We found the

binding of *An.stephensi* TEP1 to oocysts, especially to melanized oocysts, and ingestion of the anti *An.stephensi* TEP1 antibody could decrease the activity of Po enzyme, and the melanization rate of antibody treated mosquito is lower than the control mosquito. Our results suggest that nitroquine would induce the *Plasmodium* to be recognized by TEP1, and the recognition may trigger the following melanotic encapsulation.Those results suggest that the correlation of *An.stephensi* TEP1 to the melanotic encapsulation induced by antimalarial-drug nitroquine, either directly by participating in capsule formation as proteins cross linked with melanin or indirectly by triggering the activation of serine protease cascades that activate PPOs. *An. dirus* is refractory to a rodent malaria *Plasmodium yoeli* melanized oocysts are manifested in the infected mosquitoes. In this study, we cloned fragments from four PPO genes. Among the four PPO genes, the expression of *AdPPO4* was abundant in hemocyte preparation, and the gene was responsive to *P. yoelii* infection. The depletion of PPOs by RNA interference increased the *P. yoelii* infection prevalence and oocyst intensity, and abolished the melanization of oocysts as well. From the data obtained in the study, we conclued that PPO(s) participated in a mechanism that lead to the parasite elimination, and this defense does not activate melanization reaction. In the late stage the oocysts that breached the early defense were killed by a mechanism that requires no melanization, and the PPO mediated melanotic encaspulation may be just a response to the dead oocysts.

## 219

### 100 MILLION USED LONG-LASTING INSECTICIDE-TREATED NETS (LNS): HOW DO WE AVERT A POTENTIAL ENVIRONMENTAL CRISIS IN AFRICA?

**Ralph H. Rack**, Lisa Hare, Stephanie Guillaneux

*John Snow Inc., Arlington, VA, United States*

International efforts to scale up malaria control have greatly expanded LN coverage with over 48,000,000 LNs distributed last year and an estimated 250,000,000 additional LNs to be distributed by 2010. The effectiveness of LNs relies on their replacement after three to five years depending on the type of LN used. Given program targets for the next five years, Africans will be throwing away 100 million used LNs each year. Before we face a foreseeable environmental crisis, with vast amounts of insecticide and plastic polymers waste, the global public health community needs to start thinking about net collection and redistribution initiatives. There are no easy answers to this problem. A demonstration project of environmentally-sound management (ESM) of LNs, a WHO-led initiative, is currently underway to explore options to reduce the potential impact of expired nets on the environment and develop a plan for the distribution and collection of used nets to promote a life-cycle approach to product management. In addition, a number of LN manufacturers are exploring options for identifying and tracking LNs to ease collection and redistribution including color coding and bar coding of LN tags. They are also looking at limiting the environmental impact of net distribution by developing biodegradable bags and non-bag distribution options. This presentation will share preliminary findings from the demonstration project and highlight other initiatives that may provide options for limiting the adverse environmental impact of this life-saving intervention.

## 220

### QUICK AND SIMPLE DIAGNOSTIC METHOD FOR THE IDENTIFICATION OF *ANOPHELES GAMBIAE* AND *ANOPHELES ARABIENSIS* MOSQUITOES BASED ON THE LOOP-MEDIATED ISOTHERMAL PCR (LAMP)

**Mariangela Bonizzoni**[1], Yaw Afrane[2], Anne Vardo-Zalik[1], Guiyun Yan[1]

*[1]University of California Irvine, Irvine, CA, United States, [2]Center for Vector Biology and Control, Kisumu, Kenya*

The *Anopheles gambiae sensu latu* species complex comprises seven sibling species that are morphologically indistinguishable, but different in their ability to vector malaria, in their host feeding preferences, breeding requirements and geographic distributions. *An. gambiae* s.s. and *An. arabiensis* are the principal malaria vectors, they often occur in sympatry but exhibit dramatic differences in resistance to desiccation, larval habitat requirement, vectorial capacity, and responses to the application of insecticide-treated bed nets (ITNs). Ecological and environmental conditions along with a differential application of ITNs may induce a shift in malaria vector species composition, altering the vectorial system and thus malaria transmission potential. It is therefore important to assess vector species identity for understanding malaria transmission system and evaluation of vector control measures. To date, several cytological, biochemical and molecular methods have been developed to distinguish between *An. gambiae* s.s. and *An. arabiensis*, but they all require expensive laboratory equipments and can be laborious. In this study, we develop a novel species diagnostic method which may be applied under field settings in Africa, using the loop-mediated isothermal amplification (LAMP) technique. LAMP is a one step nucleic acid amplification that relies on autocycling strand-displacement DNA synthesis. It is performed under isothermal conditions (e.g., in a water bath), and the amplification products can be visualized directly by eye. Studying wild caught *An. gambiae s. l.* mosquitoes, we found that the LAMP method is as sensitive and specific in assigning *An. gambiae s.s.* and *An. arabiensis* as the rDNA-PCR method. We are currently extending the LAMP method for knockdown resistance (kdr) mutation and *Plasmodium falciparum* sporozoite detection.

## 221

### COMBINING INDOOR RESIDUAL SPRAYING AND INSECTICIDE TREATED NET INTERVENTIONS

**Immo Kleinschmidt**[1], Christopher Schwabe[2], Murugasampillay Shiva[3], Jose Luis Segura[2], Victor Sima[4], Samuel J. Mabunda[5], Michael Coleman[6]

*[1]London School of Hygiene and Tropical Medicine, London, United Kingdom, [2]Medical Care Development International, Silver Spring, MD, United States, [3]World Health Organisation, Geneva, Switzerland, [4]Ministerio de Sanidad y Bienestar Social, Malabo, Equatorial Guinea, [5]Ministerio da Saude, Maputo, Mozambique, [6]Liverpool School of Tropical Medicine, Liverpool, United Kingdom*

Does scaling up of malaria control by combining Indoor Residual Spraying (IRS) and Long Lasting Insecticidal Nets (LLIN) enhance protection to populations? Results from a literature search and from recent household surveys in Bioko, Equatorial Guinea, and Zambezia, Mozambique are presented. Five out of eight previous studies reported reduced risk of infection in those protected by both interventions compared to one intervention alone. Surveys in Bioko and Zambezia demonstrated strong evidence of a protective effect of IRS combined with nets relative to IRS alone (Odds ratio (OR) =0.71, 95% CI 0.59-0.86 for Bioko, and OR=0.63, 95% CI 0.50-0.79, for Zambezia). The effect of both interventions combined, compared to those who had neither, was OR = 0.46 [95% CI 0.76-0.81] in Bioko and 0.34[95% CI 0.21-0.56] in Zambezia. Although the effects of confounding cannot be excluded, these results provide encouragement that the additional resources for combining IRS and LLIN are justified.

## 222

### LONG-LASTING INSECTICIDAL MOSQUITO NET USAGE IN EASTERN SIERRA LEONE - THE SUCCESS OF FREE DISTRIBUTION

**Sibylle Gerstl**[1], Sophie Dunkley[2], Ahmed Mukhtar[2], Peter Maes[3], Martin De Smet[3], Samuel Baker[4], Jacob Maikere[3]

*[1]Médecins Sans Frontières UK, London, United Kingdom, [2]Médecins Sans Frontières Brussels, Bo, Sierra Leone, [3]Médecins Sans Frontières Brussels, Brussels, Belgium, [4]Ministry of Health and Sanitation, Freetown, Sierra Leone*

In eastern Sierra Leone, Médecins Sans Frontières (MSF) has run a malaria control project that includes mass distribution of free, long-lasting insecticidal mosquito nets (LNs) with demonstration of correct use. In 2006 and 2007, more than 65 000 LNs were distributed, targeting children under five years and pregnant women. The aim of this survey was to measure utilization and coverage and of LNs in the population. Heads of 900 randomly selected households in 30 clusters were interviewed regarding LNs in their household using a standardized questionnaire. The condition of the LNs in the household was also checked. Of the 4997 study persons, 67.2% (3356, CI 59.1-74.3) reported sleeping under an LN the night before the study took place. This included 76.8% (926/1206, CI 69.8-82.6) of children under five and 73% (100/137, CI 59.8-83.1) of pregnant women. Of the 900 households, 751 (83.4%, 95%CI 78.5-88.4) reported owning at least one LN. Of the 16.6% who did not own an LN (149/900, CI 11.6-21.5), 91.9% did not participate in the MSF LN mass distribution. In the 751 households reporting LNs, 94.1% (707/751) were observed to have them hanging correctly over the bed. Of the 1135 correctly hanging LNs, 52.7% (598/1135) did not have any holes and another 22.6% (256/1135) had less than 10 finger size holes. The main reasons for not hanging LNs were that they were currently not yet used/still in original packaging (38.2%, 109/286) or that the LNs were used to wrap the mattress as bedbug protection (34.6%, 99/286). The average age of the LNs was two years and 99% were washed less than 20 times. The most common source of LNs was MSF (75.2%). Our study indicated that MSF achieved very good coverage with LNs in the catchment area. It is one of the few areas where utilization results surpassed the targets set in 2000 by Roll Back Malaria, namely having at least 60% of pregnant women and children under five using LNs. The condition of the LNs was also in line with recommendations from the WHO for washing and duration of usage.

## 223

### A NEW ANTI-MOSQUITO INSECTICIDE PAINT: EVALUATION OF ITS FIELD EFFICACY AGAINST *ANOPHELES GAMBIAE* AND *CULEX QUINQUEFASCIATUS* OVER 12 MONTHS

Beatriz Mosqueira[1], J. Chabi[2], Fabrice Chandre[2], Martin Akogbeto[3], Jean-Marc Hougard[3], Pierre Carnevale[2], **Santiago Mas-Coma**[1]

*[1]Departamento de Parasitologia, Universidad de Valencia, Burjassot - Valencia, Spain, [2]UR016, Institut de Recherche pour le Developpement, Montpellier, France, [3]Centre de Recherche Entomologique de Cotonou, Cotonou, Benin*

Malaria remains a major public health concern in West Africa. The increasing resistance of anopheline vectors to insecticides suggests the need for new strategies against malaria vectors. We studied the efficacy of an insecticide paint containing two organophosphates and one IGR, against pyrethroid resistant populations of *Anopheles gambiae* and *Culex quinquefasciatus* in experimental huts in Cotonou, Benin. In early morning collections, the twelve-month trial showed an overall mortality of 90-100% for up to 6 months. 9 months after treatment, rates remained high (65-75%) in huts painted with two layers. 12 months after treatment, mortality rates were still significant, but below operational levels. No deterrent or excito-repellent effect was observed. In the WHO 30-minute bioassays, a residual efficacy of 100% on all huts against *Cx. quinquefasciatus* was observed after 6 months. 100% mortality rates were observed in *An. gambiae*. By 12 months, residual efficacy of 60-80% was observed in both species. Mosquito-release experiments performed at T0 showed reductions in blood-feeding of 82-97% on treated huts. In the distance tests, mortality at distances of 1 m was 96-100% in both species for up to 12 months. The lethal effect observed in both species is noteworthy. Killing was not only high but also quick enough to reduce blood-feeding even in the absence of a physical barrier. Resuts obtained in the distance tests point at a possible mass effect. In view of these promising results, further research is being done on the profile of this product. In addition to the efficacy observed, the insecticide paint has operational advantages over long-lasting impregnated nets and tools like indoor residual spraying, because no training is needed for its application or day to day use and homes would gain in appearance and, more importantly, hygiene. Given the nature of the product and characteristics surrounding African cities, it appears that urban areas and public buildings could benefit from the protection conferred by the paint.

## 224

### PHARMACOKINETICS (PK) AND PHARMACODYNAMICS (PD) AND SAFETY OF ARTESUNATE (AS) AND DIHYDROARTEMISININ (DHA) FOLLOWING A SINGLE ORAL DOSE OF ARTESUNATE DURING THE 2ND AND 3RD TRIMESTER OF PREGNANCY

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

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

Safety and pharmacokinetic data supporting the use of Artemisinin-based combinations in pregnancy are urgently needed. Because of a lack of these data, in many countries, Sulfadoxine-pyrimethamine is still used for intermittent presumptive treatment of malaria during pregnancy despite a high level of resistance. We hypothesized that there are lower levels of AS and DHA in pregnant women compared to controls, particularly during the third trimester. A 200 mg oral dose of AS was administered to pregnant women with falciparum malaria infection between 22-26 weeks (n=13) and 32-36 weeks gestation (n=13). Venous blood samples were drawn at 0, 15, 30, 45, 60 and 90 minutes and 2, 3, 4, 6 and 8 hours after dosing for PK determination; capillary blood was collected at 0 minute, 12, 24, 30, 36, 42, and 48 hours for PD determination. For control data, blood sampling was repeated at 3 months postpartum in these subjects and in 25 non-pregnant women (external control). PK samples were centrifuged immediately and plasma was stored at -80 $^{0}$C before being analyzed for total and free levels of AS and DHA, using liquid chromotography-mass spectroscopy. The distributions of parasite density were comparable in pregnant and external control groups (median [min, max]: 528 [211, 3420] vs 807[208, 32262] parasites per µL, p=0.21). There were no stillbirths and no congenital abnormalities. The median birth weight was 3025 (2130, 3620) grams; 2 (7.7%) infants weighed ≤2500 gms. There were no adverse events related to the study drug. Conventional non-compartmental modeling on the total and free AS and DHA levels are being performed and the results will be presented.

## 225

### IMPACT OF BEDNET USE, INTERMITTENT PREVENTIVE TREATMENT (IPT), AND ANTENATAL CARE (ANC) DURING PREGNANCY ON THE HEALTH OF NEWBORNS IN THE KASSENA NANKANA DISTRICT OF NORTHERN GHANA

**David J. Fryauff**[1], Frank Atuguba[2], Kwadwo A. Koram[3], Thomas Anyorigiya[2], Martin Adjuik[2], Abraham R. Oduro[2], Patrick Ansah[2], Abraham Hodgson[2], Lucas Amenga Etego[2], Francis Nkrumah[3]

*[1]Naval Medical Research Center, Silver Spring, MD, United States, [2]Navrongo Health Research Center, Navrongo, Ghana, [3]Noguchi Memorial Institute of Medical Research, Accra, Ghana*

Primigravid women and their first-born babies are especially vulnerable to malaria but health practices during pregnancy can give mother and baby better defense against disease and longer malaria-free periods in which to grow and develop. We focused upon infants born of first-time mothers to analyze for protective effects that may result from IPTp coverage, ANC attendance, and/or bednet use during pregnancy. Primigravid mothers gave birth more often to low birth weight (LBW) infants than multigravida mothers (26% vs 16%; $p < 0.0001$). Near equal proportions of LBW girls and boys were born to first-time mothers, but among multigravid mothers, the majority of LBW babies were girls (24% vs. 13%; $p = 0.01$). Primigravid mothers averaged 9 years younger than multigravid mothers but these younger mothers had significantly more education, more ANC attendance, and better IPTp compliance. Despite lower birth weights of infants born to first time mothers, these children had healthier Hb profiles and significantly less mild anemia (Hb < 11.0). Relative to primigravid mothers who reported no IPTp during pregnancy (86/551=16%), just one IPTp dose of Fansidar (114/551=21%) was associated with a significantly greater mean birth weight, a significantly lower proportion of LBW newborns, an improved Hb profile, and fewer mild anemias. In contrast to the positive effects seen in primigravid mothers, analysis of a larger sample of multigravid mothers revealed no significant or even marginal improvements in birth weights or hemoglobin associated with one dose of Fansidar. We found no benefit to newborns that could be associated with bednet use during pregnancy in either primigravid or multigravid mothers who reported no IPTp use. Bednet use during pregnancy was associated with some notable differences in mothers: older age, more education, more ANC visits, better IPTp coverage, more frequently reported illness, and a history of miscarriage or the death of a child. Our findings suggest that first time mothers and their babies may benefit from even minimal IPTp use during pregnancy.

## 226

### FACTORS AFFECTING MALARIA PREVENTION AND TREATMENT DECISIONS FOR CHILDREN IN THE DEMOCRATIC REPUBLIC OF CONGO

**Olufunke A. Alaba**[1], Gauthier Tshiswaka Kashalala[1], Adefolarin A. Fawole[2]

*[1]University of Pretoria, Pretoria, South Africa, [2]CentaMed Practitioners, Pretoria, South Africa*

Though endowed with natural resources with the country is one of the poorest countries of the world with a Human Development Index of 0.43; ranking 152nd out of 175 countries. The economic, political and war crises in the Democratic Republic of Congo (DRC) has negatively impacted on public health risks for the population. In recognition of the deep crisis, the government of DRC is thereby, promoting expansionary strategies to combat malaria as an important component in the global strategy to fight poverty and improve the standard of living of the people. Malaria is one of the primary causes of mortality and morbidity in the country, especially among pregnant women and young children, accounting for 30% of child mortality. However, insofar the government has come up with various control strategies including appropriate case management in both community and health infrastructures, scaling up the use of insecticide treated nets (ITNs) among others, it is also necessary to think beyond supply. Specifically, we need to consider how family take decisions on behalf of the children during episodes of malaria and what social, economic and environmental factors affect this behaviour. Therefore, the main objective of this paper is to provide quantitative and qualitative evidence on the importance of individual, household and community characteristics on care seeking decisions during episodes of malaria in children as well as factors affecting malaria prevention methods in the DRC. The analyses will be based on the DRC Multiple Indicator Cluster Survey, (MICS) of 2001 supported by UNICEF. It is expected that the results will shed more light on the demand component associated to the prevention and treatment of malaria in this war-torn country.

## 227

### EFFICACY AND WASH RESISTANCE OF α-CYPERMETHRIN WHO RECOMMENDED LONG LASTING INSECTICIDE NET AGAINST *ANOPHELES DARLINGI* IN THE PERUVIAN AMAZON

Elvira Zamora[1], Daniel Del Cuadro[1], **Hugo Rodriguez**[2], Gregor J. Devine[3], Yuri Alegre[1], Angel Rosas[2]

*[1]Regional Direction of Health Loreto, Iquitos, Peru, [2]Multi-Country Malaria Project PAMAFRO - Organismo Andino de Salud - Convenio Hipolito Unanue, Lima, Peru, [3]Rothamsted Research, Harpenden, United Kingdom*

The Peruvian Malaria Program has adopted Long Lasting Insecticide Net (LLIN) distribution as important strategy to prevent and control malaria in high risk areas of malaria transmission in the Amazon Region. The Interceptor® net, manufactured by BASF, is an α-cypermethrin treated LLIN that received WHOPES interim recommendation after an accelerated wash-resistance and small scale field-testing against susceptible African mosquitoes, mainly *An. gambiae*. It is necessary to confirm the efficacy of this LLIN against *Anopheles darlingi*, the most common and efficient malaria vector in the Peruvian Amazon. The objective of this study was to determine the efficacy and wash resistance of Interceptor®, under laboratory conditions, against *Anopheles darlingi* in Zungarococha, San Juan, Loreto-Peru. Mosquitoes were collected the night before each bioassay. According to the WHO guidelines for laboratory testing, four different Interceptor® nets were tested without washing, and then with standard numbers of washes (1, 5, 10, 15 and 20 times). Susceptible *Anopheles darlingi* mosquitoes were exposed to net samples (25 cm x 25 cm) for 3 minutes using standard WHO cones. 50 mosquitoes per net were used (10 replicates). After exposure they were maintained in clean cups with access to sugar solution. Knock down effect (KD) was measured after 60 minutes post-exposure and mortality after 24 hours. A LLIN meets the WHO criteria when KD>95% and/or mortality>80% after 20 washes. Interceptor® net caused moderate KD and mortality of *An. darlingi* before washing (KD = 83.0%; mortality = 49.0%), but after first wash efficacy improved (KD = 92.0%; mortality = 79.0%). Efficacy then decreased according to the number of washes: 5 times (KD = 97.0%; mortality = 76.0%), 10 times (KD = 87.0%; mortality = 68.5%), 15 times (KD = 87.5%; mortality = 64.0%) and 20 times (KD = 80.0%; mortality = 62.5%). In conclusion, Interceptor® against susceptible *Anopheles darlingi* from Zungarococha (San Juan) had a moderate efficacy without and with washing, and did not meet the WHO criteria.

## 228

### ACCEPTABILITY AND INITIAL USE OF LONG-LASTING INSECTICIDE NETS (LLIN) AS MEASURE TO PREVENT AND CONTROL MALARIA IN COLOMBIAN BORDER AREAS

Edison Soto, Andres Cuervo, Angel Rosas, **Daniel Vargas**, Lina Florez, Luis Valera

*Multi-Country Malaria Project PAMAFRO - Organismo Andino de Salud - Convenio Hipolito Unanue, Lima, Peru*

With the support of the Global Fund's Multi-Country Malaria Project PAMAFRO, Colombian Malaria Program has incorporated the Long Lasting Insecticide Net (LLIN) distribution as important strategy to prevent and control malaria in 5 departments that share borders with Andean

Region countries (Arauca, Guainía, Nariño, Putumayo and Vichada). From April to October 2007, 41.978 Olyset® LLINs were delivered in 229 localities, covering 10.581 households and 71.105 citizens. This study was undertaken to determine the acceptability and initial use of the LLINs as measure to prevent and control malaria in Colombian border areas. Following the introduction of LLIN (3 to 6 months after), a cross-sectional community-based survey was undertaken in 1.973 households from 90 localities, using a structured questionnaire, to assess the demographic variables relevant to LLIN use, acceptability of LLIN and net adverse reactions. Most of people reported to have slept under the net the previous night (92,1%). General LLIN acceptability was high (96,3%), however some specific characteristics of LLIN did not have high acceptability as the too big mesh size (72,1%), mainly in Amazon departments. There were only temporary mild adverse reactions (3%) after use of LLINs, mainly body itch. In conclusion, LLINs were widely acceptable in the user communities of Colombian border areas and their initial use was very high. We will continue promoting and monitoring appropriate LLIN use having into account the local needs and preferences.

## 229

### THE IMPACT OF ANTIMICROBIAL PEPTIDE KNOCK DOWN BY RNA INTERFERENCE ON LONGEVITY AND REPRODUCTIVE OUTPUT IN *AEDES AEGYPTI*

**Raul Ursic Bedoya**, Courtney Mckay, Carl Lowenberger

*Simon Fraser University, Burnaby, BC, Canada*

*Aedes aegypti* is a major vector of arboviruses in the tropics and has become an important research organism to study insect-virus interactions. Insects, including mosquitoes, have a potent innate immune system to eliminate invading microorganisms. Cellular immunity is assumed by hemocytes, which phagocytose small microorganisms. Humoral defenses are characterized by a large number of potent antimicrobial peptides, reactive intermediates of nitrogen or oxygen and a complex enzymatic cascade yielding in clotting or hemolymph melanization. To evaluate the role of humoral defenses in *Aedes aegypti*, we silenced the expression of three different antimicrobial molecules (cecropin, bomanin and lysozyme) using RNA interference and assessed the effects of the knock down on longevity and reproductive output in response to bacterial infection. Gene silencing was verified by real time PCR. Our results show no significant difference in mortality or reproductive output when cecropin, bomanin or lysozyme are knocked down. The data supports the notion that antimicrobial molecules function as a later component of the insect immune response.

## 230

### HUMAN INSULIN INGESTED BY *ANOPHELES STEPHENSI* MOSQUITO SIGNALS IN THE MIDGUT TO INCREASE OXIDATIVE DAMAGE AND REDUCE LIFESPAN

**Nazzy Pakpour**, Laura Dickson, Kong Cheung, Shirley Luckhart

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

*Plasmodium* parasites, the causative agent of malaria, are transmitted through the bites of infected *Anopheles* mosquitoes and take up to two weeks in the mosquito to develop into the infectious sporozoite form. The potential exists to disrupt this development by enhancing the mosquito's innate immune system and/or by shortening the lifespan of the mosquito. The insulin/IGF-1 signaling (IIS) cascade can regulate both innate immunity and lifespan in a variety of organisms including *Caenorhabditis elegans* and *Drosophila melangaster*. We have previously shown that ingested human insulin leads to the activation of Akt in the mosquito midgut, a downstream signaling molecule of the IIS cascade. We have also shown that mosquitoes fed bloodmeals containing human insulin died at a faster rate than blood fed controls. In mammals, the reduction in lifespan observed following induction of the IIS cascade has been linked to increased oxidative damage that occurs due to increased synthesis of reactive oxygen species and down-regulation of antioxidants. We show here that *An. stephensi* mosquitoes fed bloodmeals containing human insulin had increased phosphorylation of the transcription factor FOXO, which regulates production of antioxidants, such as manganese superoxide dismutase (MnSOD). Additionally, mosquitoes fed bloodmeals containing human insulin on a weekly basis accumulated higher levels of oxidative damage, as determined by total protein carbonyl content. This increased oxidative damage could be reduced by the inclusion of a cell-permeable SOD mimetic in the bloodmeal. Taken together, our data suggests that the IIS induces the synthesis of reactive oxygen species while down regulating antioxidants, leading to increased oxidative damage and a reduction in lifespan. Therefore, manipulation of the IIS cascade in *Anopheles* mosquitoes could be a valid method for reducing the vectorial capacity of these mosquitoes.

## 231

### USING ENHANCED REPRODUCTION AS A NOVEL DRIVE MECHANISM FOR MOSQUITO POPULATION REPLACEMENT

**Anam Javed**[1], Jason L. Rasgon[2], Kendra M. Quicke[1], Michael A. Riehle[1]

*[1]University of Arizona, Tucson, AZ, United States, [2]Johns Hopkins University, Baltimore, MD, United States*

A promising proposal for controlling mosquito-borne diseases is to replace the wild mosquito populations with ones incapable of transmitting disease, such as dengue or malaria. A key step towards implementing population replacement is to devise a mechanism to drive effector genes into natural mosquito populations. We propose that increased reproduction could effectively drive transgenic mosquitoes through a wild population. In *Aedes aegypti*, the primary vector of dengue fever and dengue hemorrhagic fever (DHF), and other mosquitoes the insulin/ insulin growth factor I signaling (IIS) cascade is a key regulator of many physiological processes, including reproduction. Using RNAi, we knocked down expression of the IIS inhibitor, PTEN, leading to increased insulin signaling. Knockdown of AaegPTEN or its specific splice variant AaegPTEN6 (the splice variant responsible for regulating reproduction in the ovary and fat body) led to a 15-63% increase in egg production and up to a 32% increase in egg viability during the first reproductive cycle. Knockdown of AaegPTEN3, expressed predominantly in the head, had no effect on reproduction. Modeling data demonstrated that as little as a 10% increase in reproduction would lead to population replacement within approximately 100 generations; larger fitness benefits lead to population replacement in correspondingly shorter time periods. Our results suggest that increasing reproduction through the manipulation of the IIS cascade could be a novel strategy for driving transgenic mosquitoes into wild populations.

## 232

### FUNCTIONAL ANALYSIS OF TAK1 AND IAP2 IN THE YELLOW FEVER MOSQUITO, *AEDES AEGYPTI*

**Shin-Hong Shiao**

*National Taiwan University, Taipei, Taiwan*

It has long been recognized that insects mount a powerful and efficient immune system to fight against invading pathogens. The insect immune response is of cellular and humoral nature, but it lacks adaptive features. They make use of two distinct signaling pathways: the Toll pathway and IMD (immunodeficiency) pathway to mount diverse antimicrobial activities. Here, we identified two components of the IMD pathway, namely inhibitor of apoptosis 2 (IAP2) and transforming growth factor-activated kinase 1 (TAK1) in the yellow fever mosquito *Aedes aegypti*. The transcripts of both IAP2 and TAK1 were analyzed by Real-Time PCR and were increased after *E. coli* challenge. Silencing of IAP2 and TAK1 significantly decrease the survival rate upon *E.coli* but not *S. aureus* challenge, suggesting that both IAP2 and TAK1 involved in the expression of IMD-dependent antimicrobial peptides. The translational expression of IAP2 was increased after *E. coli* challenge over time. Tissue specific expressional analysis were examined

and showing that IAP2 were highly expressed in the mosquito fatbody and midgut in terms of transcriptional and translational level. Transcriptional patterns of two antimicrobial peptides Cecropin A and Defensin A were examined. Preliminary results showed that the induction of both Cecropin A and Defensin A were significantly reduced in IAP2 and TAK1 knockdown mosquitoes after *E. coli* challenge. Information gathered in this study will pave the way toward the establishment of efficient strategies for vector control using molecular engineering approaches.

## 233

### WEST NILE VIRUS INFECTION ALTERS MIDGUT GENE EXPRESSION IN *CULEX PIPIENS QUINQUEFASCIATUS* SAY (DIPTERA: CULICIDAE)

**Chelsea T. Smartt**[1], Stephanie L. Richards[1], Sheri L. Anderson[1], Jennifer S. Erickson[2]

*[1]University of Florida, Vero Beach, FL, United States, [2]McMaster University, Hamilton, ON, Canada*

Alterations in gene expression in the midgut of female *Culex pipiens quinquefasciatus* exposed to blood meals containing 6.8 logs plaque-forming units/mL of West Nile virus (WNV) were investigated by Fluorescent Differential Display. Twenty-six different cDNAs exhibited reproducible differences after feeding on infected blood. Of these, 21 cDNAs showed an increase in expression and five showed a decrease in expression as a result of WNV presence in the blood meal. GenBank database searches revealed that one clone with increased expression, CQ G12A2, shares 94% identity with a leucine-rich repeat-containing protein from *Cx. p. quinquefasciatus* and 32% identity to Toll-like receptors from *Aedes aegypti*. We present the first cDNA clone isolated from female *Cx. p. quinquefasciatus* midgut tissue whose expression changes upon exposure to WNV. This cDNA represents a mosquito gene that is an excellent candidate for interacting with WNV in *Cx. p. quinquefasciatus* and may play a role in disease transmission.

## 234

### ESTABLISHMENT OF AND EVALUATION ON A STABLY EXPRESSING AND KNOCKDOWN SYSTEM IN MOSQUITO CELL LINES

**Chao-Fu Yang**, Wei-June Chen

*Chan Gung University, Tao-Yuan, Taiwan*

Mosquito is important for transmission of a variety of infectious diseases, such as malaria, dengue fever, yellow fever, etc, particularly in tropical and subtropical countries. The interaction between mosquito and mosquito-borne pathogen is still poorly understood now, especially at molecular level. Many kinds of expression vectors have been reported to be useful for expression of interesting genes in mosquito cell lines. Most of them are virus-based, such as *Aedes aegypti* densovirus, recombinant vaccinia virus, and sindbis virus expression vector. One of major drawbacks in virus-based expression vectors is that virus-based vector effects are hardly excluded. Although some plasmid-based expression vectors have also been used in studying mosquito, they are not easy to be applied due to lower efficiency of transient transfection in mosquito cell lines. In this study, we have established a plasmid-based stable expression and knockdown system in mosquito cell lines. We incorporated *Drosophila* actin 5C promoter and baculovirus *ie1* promoter into the plasmid to drive antibiotic resistant gene and target gene, respectively. We used this vector to express either eGFP or C189-eGFP fusion protein in C6/36 cells, which stable expression clone have been successfully acquired after selecting by the antibiotic G418. Then, we combined murine miR-155 flanking sequences with this stable expression vector to generate a miRNA-based stable knockdown system, which successfully decreased endogenous C189 gene expression at mRNA and protein level in C6/36 cells. This system is believed to be a novel strategy in stably expressing or knockdown specific genes within mosquito cells.

## 235

### MOSQUITO TRANSCRIPTOME CHANGES AND FILARIAL WORM SUSCEPTIBILITY IN *ARMIGERES SUBALBATUS*

**Matthew T. Aliota**, Jeremy F. Fuchs, Thomas A. Rocheleau, Bruce M. Christensen

*University of Wisconsin, Madison, WI, United States*

*Armigeres subalbatus* is a natural vector of the filarial worm *Brugia pahangi*, but it kills *B. malayi* mf by melanotic encapsulation. Because *B. malayi* and *B. pahangi* are morphologically and biologically similar, comparing Ar. subalbatus-*B. pahangi* susceptibility and Ar. subalbatus-*B. malayi* refractoriness could provide significant insight into recognition mechanisms required to mount an effective anti-filarial worm immune response in the mosquito, as well as provide considerable detail into the molecular components involved in vector competence. Accordingly, we have initiated transcriptome profiling studies of Ar. subalbatus in relation to filarial worm infection to clarify molecular components involved in *B. pahangi* susceptibility. Volcano plots were used to create working gene lists to identify differentially expressed transcripts at each time point. At 1, 3, 6, 12, 24 h PI and 2/3, 5/6, 8/9, and 13/14 d post challenge there were 33, 75, 113, 76, 54, 5, 3, 13, and 2 detectable transcripts, respectively, with significant differences in transcript abundance (increase or decrease) as a result of parasite development. The time course chosen facilitated an examination of key events in the development of the parasite, beginning with the very start of filarial worm infection and spanning to well after infective stage parasites had developed in the mosquito. Herein, we demonstrate that filarial worm susceptibility in a natural vector, such as Ar. subalbatus, is a highly complex process during the first 24 hours of infection that involves many factors of both known and unknown function that most likely are associated with filarial worm penetration through the midgut lumen, invasion into thoracic muscle cells, and maintenance of homeostasis in the hemolymph environment. This is in contrast to what is reported in the *Aedes aegypti-Brugia malayi* model of infection (a laboratory selected model), where the greatest change in the mosquito's transcriptome was associated with infective stage parasites in the mosquito's head and thorax.

## 236

### A SHUT-DOWN IN EXPRESSION OF CATALASE INCREASES OVARIAN APOPTOSIS DURING THE OVERWINTERING DIAPAUSE OF THE MOSQUITO *CULEX PIPIENS*

**Cheolho Sim**, Denlinger L. David

*Department of Entomology, The Ohio State University, Columbus, OH, United States*

*Culex pipiens*, the mosquito that vectors West Nile virus in North America, overwinters in an adult diapause (dormancy) that is programmed by the short day length of autumn. In response to this environmental signal, insulin signaling is shut down and forkhead transcription factor (FOXO), in turn, initiates the induction of genes involved in the diapause syndrome, including stress tolerance and extended lifespan. It is well known that oxidoreductases increase stress tolerance and extend the lifespan of many organisms, but a link with diapause has not previously been examined. We hypothesize that oxidoreductase genes have a significant role in stress tolerance and in extending the lifespan during diapause, a response that may be regulated by FOXO through the insulin signaling pathway. In this study, five oxidoreductase genes including superoxide dismutases, catalase and glutathione peroxidase genes were identified. Expression of *superoxide dismutase* and *catalase* was elevated in females during early diapause. A systemic reduction in catalase activity by dsRNA-mediated knockdown significantly increased apoptosis in follicles that were developmentally arrested in diapause, indicating that catalase plays an important role in protecting the ovaries from stress during diapause.

## 237

### HOW DOES *PLASMODIUM FALCIPARUM* EVADE OF THE MOSQUITO IMMUNE SYSTEM

**Alvaro Molina-Cruz**, Corrie Ortega, Jared Winikor, Janneth Rodrigues, Carolina Barillas-Mury

*National Institutes of Health, Bethesda, MD, United States*

*Plasmodium* parasites can be detected and eliminated by the mosquito innate immune system. The refractory *Anopheles gambiae* L35 strain was used to study the mosquito antiparasitic responses and to map the *P. falciparum* gene(s) that determine parasite survival in the mosquito. The L35 strain is susceptible to a *P. falciparum* line from Africa but highly refractory to a line from Brazil, in which more than 98% of the parasites are melanized. Silencing experiments revealed that melanization of the Brazilian strain in L35 females is mediated by the TEP1/LRIM1/APL1 (LRIM2) complex. A coinfection of African and Brazilian strains was done to determine whether systemic activation of the mosquito immune response was sufficient to kill both *P. falciparum* strains. Coinfection gave rise to a mixed phenotype, with live oocysts and melanized parasites present on individual midguts; indicating that, although the mosquito immune system is activated and some parasites are killed through activation of the TEP1/LRIM1/APL1 complex, a substantial number of parasites survive. This suggests that there are *Plasmodium* genetic factors that determine whether the parasite is able to survive the mosquito defenses. A QTL analysis (gene mapping) of a cross between an African (GB4) and a Brazilian *P. falciparum* (7G8) strains was done to identify *P. falciparum* loci determining parasite survival in the L35 strain. Phenotyping of parental and progeny lines for survival/melanization indicates that the trait has only 2 states (oocyst survival, or near to 100% melanization), with no intermediate phenotypes. Preliminary QTL analysis suggests that a single locus in chromosome 13 confers *P. falciparum* parasites of African origin the ability to evade the *An. gambiae* immune response.

## 238

### COMPARISON OF IMMUNOGENIC PROTEINS TO SALIVA OF *ANOPHELES ALBIMANUS, ANOPHELES STEPHENSI* AND *AEDES AEGYPTI* IN SERUM FROM INDIVIDUALS LIVING IN AREAS WHERE THESE SPECIES ARE OR ARE NOT ENDEMIC

**Berlin L. Londono**[1], Daisy Carvajal[2], Diva Palacios[3], Chad Massey[1], Joseph Keating[1], Thomas Eisele[1], Mark James[1], Don Krogstad[1], Dawn Wesson[1]

*[1]Tulane University, New Orleans, LA, United States, [2]Universidad de Pamplona, Pamplona, Colombia, [3]Hospital San Francisco de Asis, Quibdo, Colombia*

Antibodies against arthropod vector saliva are considered important markers of mosquito bite exposure and they are very useful in the evaluation of vector borne diseases like malaria. In our study, salivary gland extracts from *Anopheles albimanus*, *An. stephensi* and *Aedes aegypti* mosquitoes were run in pre-cast 12% Tris-Bis polyacrylamide gels and transferred to PVDF membranes. Pooled sera from individuals living in malaria endemic areas (Colombia low lands, Haiti and Guinea) and individuals living in non-malaria endemic areas (United States and Colombia high lands) were tested against the immobilized proteins. Immunogenic proteins were cut from polyacrylamide gels and submitted for amino acid sequencing. The purposes of the study were 1) To identify any cross reaction among salivary proteins from different mosquito species and/ or mosquito genera that eliciting immune response in individuals living in regions where these mosquitoes are or are not endemic and 2) To evaluate whether there are differences in the proteins recognized for antibodies in people living in holo - endemic and epidemic areas.

## 239

### THE ROLE OF SLC7-TYPE AMINO ACID TRANSPORTERS IN MOSQUITO IMMUNITY, REPRODUCTION AND LIFESPAN

**Lisa L. Drake**, Sarah Aguirre, Immo A. Hansen

*NMSU, Las Cruces, NM, United States*

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

## 240

### AN EVOLUTIONARY CONSERVED FUNCTION OF THE JAK-STAT PATHWAY IN ANTI-DENGUE DEFENSES

**Jayme A. Souza-Neto**, George Dimopoulos

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

Dengue fever has become a major arthropod-borne viral disease affecting humans and public health concern worldwide. Although major pathways such as the RNAi machinery and the Toll immune signaling cascade have been implicated in the mosquito's anti-dengue responses, to date there is no known well characterized anti-dengue effector (dengue virus restriction factor, DVRF). The JAK-STAT pathway is a conserved immune signaling in humans and insects antiviral defenses, and has been shown to be an important component of the human anti-dengue responses. Here we present an evolutionary conserved function for the JAK-STAT pathway in anti-dengue responses in the major mosquito vector of dengue virus, *Aedes aegypti*. This mosquito activates and uses the JAK-STAT pathway to control virus infection, presumably through one or several STAT-regulated DVRFs. The mosquito's susceptibility to dengue virus increases when the JAK-STAT pathway is suppressed through RNAi depletion of the positive regulator Domeless (Dome), while mosquitoes become more resistant to the virus when the pathway is activated by silencing the negative regulator, PIAS. We further identified a repertoire of JAK-STAT/dengue infection-responsive genes candidate to DVRF function using microarrays and qRT-PCR assays. Finally, an RNAi screen revealed two genes that when depleted rendered mosquitoes more susceptible to dengue virus infection, suggesting that they are part of the JAK-STAT pathway-regulated anti-dengue response. These novel identified DVRFs have STAT biding sites in their promoter region and code to secreted proteins. In summary, here we report the implication of the JAK-STAT pathway in the *A. aegypti* anti-dengue defense and the identification of two novel DVRFs. Considering the evolutionary conservation of the pathway components and function in human's and mosquito's anti-dengue responses, studies on dengue-mosquito interactions may therefore also have great relevance for the development of human therapies in addition to vector-based dengue control strategies.

## 241

### EFFECT OF *AEDES AEGYPTI* SALIVA ON MACROPHAGE CYTOKINE EXPRESSION AND ON DENGUE INFECTION

**Karine Delroux**

*SRI International, Center for Advanced Drug Research (CADRE), Harrisonburg, VA, United States*

Dengue (DEN) affects 50-100 million people worldwide every year and is particularly deadly among infected children and young adults. No antiviral drugs are available for DEN, and there is no vaccine. The only treatment is supportive care. The mosquito *Aedes aegypti* is the global vector of DEN and transmits the virus to humans through an infective bite. When a mosquito bites, it injects saliva that contains anticoagulant, vasodilatory, anti-inflammatory, and immunomodulatory compounds. By suppressing the host's response to injury, salivary proteins also facilitate viral transmission and early infection. Although *A. aegypti* saliva can shift the balance toward the Th2 immune response by suppressing Th1 cytokine expression in mouse lymphocytes, the immunomodulatory function of *Ae. aegypti* saliva has not been demonstrated in macrophages against DEN virus challenge. Macrophages are primary responders to infection, and their role is crucial in subsequently stimulating specific lymphocytes to elicit an immune response. In addition, macrophages are one of the main target cells of DEN virus. We hypothesize that *A. aegypti* saliva alters DEN infection in host cells by modulating cytokine expression. We will use multiplex immunoassay to measure the levels of Th1 and Th2 cytokine responses of human macrophages when challenged with *Ae. aegypti* saliva alone or mixed with DEN virus. We will then quantify DEN viral replication in infected cells by quantitative RT-PCR, and DEN infectivity by plaque assay. By using a proteomics approach supported by available genomic and sialomic databases, we will be able to identify the salivary proteins responsible for modulating the host cytokine profile and thereby affecting DEN virus infection.

## 242

### ACCEPTANCE AND WILLINGNESS-TO-PAY OF A NOVEL "PUSH-PULL" STRATEGY FOR *AEDES AEGYPTI* VECTOR CONTROL IN CENTRAL THAILAND

**Valaikanya Plasai**[1], Theeraphap Charoenviriyaphap[2], John Grieco[2], Nicole Achee[2]

*[1]Bureau of Vector-Borne Diseases, Ministry of Public Health, Nonthaburi, Thailand, [2]Uniformed Services University of the Health Sciences, Bethesda, MD, United States*

As part of a larger research program evaluating a push-pull strategy against *Aedes aegypti* using spatial repellent tools, the objective of this study was to determine acceptance and willingness-to-pay for a product designed to repel mosquitoes in homes *vs*. products designed to kill mosquitoes in two communities endemic for mosquito-borne disease. A purposive sampling method was used to identify two distinctly different communities in Kanchanaburi province in west-central Thailand: one dengue-endemic, the other with a long history of malaria, but no dengue in recent years. A total of 101 key informants, stratified by gender and age to include heads of households and village health volunteers, participated in 10 focus group discussions in the two sites. Participants in both sites currently practice insect control strategies that indicate they are familiar with the concept of spatial repellency (*i.e.,* the use of electric fans, *etc*.). Even though participants in the dengue-endemic community reported a significantly higher and more frequent use of insecticide products (6 to 24 cans of spray per year) than those living in the malaria-endemic community (1 can per year), both are concerned of the potentially harmful effects of aerosolized insecticides and are eager to stop their use if equally effective alternatives are available. Both communities expressed keen interest in trying the novel strategy, and were willing to pay for it if it cost no more than what they normally spent annually for mosquito control, *i.e*., a maximum of THB2,500 per year (USD73.5) in the dengue-endemic community, and THB100 (USD3) in the malaria-endemic area. The reason for this difference may be simply economic, given that the malaria-endemic community represents a much lower economic bracket. Even so, we conclude that household-based strategies designed to reduce man-vector contact using spatial repellency are and will be accepted in Kanchanaburi, although we have not proven that the motivation is disease prevention rather than the reduction of mosquito nuisance. Results from this study are instrumental in guiding the experimental design for the overall program and furthering the development of a sustainable approach.

## 243

### LARVAL TEMPERATURE AND NUTRITION ALTER THE SUSCEPTIBILITY OF *AEDES AEGYPTI* MOSQUITOES TO CHIKUNGUNYA VIRUS

**Catherine J. Westbrook**, L. Philip Lounibos

*Florida Medical Entomology Laboratory, Department of Entomology and Nematology, University of Florida, Vero Beach, FL, United States*

Variations in the larval environment can influence adult mosquito traits that in turn affect vector competence. To explore relationships between larval temperature, nutrition, and vector susceptibility we reared *Aedes aegypti* larvae at 20, 27 and 34°C and with two diet levels and then orally infected adult females with a blood meal containing 6.3 $Log_{10}$ PFU/ml of chikungunya virus, which recently emerged and re-emerged as a globally important human arboviral pathogen. We recorded development time to adulthood, wing length, the presence or absence of a chikungunya midgut infection and disseminated infection, and body viral titer. We found that larval temperature, but not diet, nor the temperature X food interaction, had a significant effect on chikungunya infection, but that both diet and the interaction had a significant effect on dissemination, while temperature did not. We also measured body titer and wing length from a subset of freshly engorged mosquitoes from each temperature-diet treatment to determine if the amount of virus ingested from the infectious blood meal was correlated with wing size. We found that wing length was positively correlated with the initial amount of virus ingested, but significant wing length - infection correlations disappeared after the extrinsic incubation period. This study suggests that larval environmental variables are important in shaping vector-viral interactions and that mosquito size alone may not be a good predictor of viral susceptibility.

## 244

### INSECTARY MAINTENANCE OF ASIAN MALARIA VECTORS: AN EVALUATION OF THE EFFECTS OF BLOODMEAL SOURCE ON FEEDING, MORTALITY, FECUNDITY AND EGG HATCHING RATES

**Siriporn Phasomkusolsil**

*Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand*

The artificial membrane feeding technique has replaced direct feeding on animals for the maintenance of malaria and arbovirus vector in many laboratories. Membrane feeding also facilitates controlled experimentation of pathogen transmission during mosquito feeding. Sheep blood is commonly used because of its availability and low cost. Here we compared membrane feeding on sheep, guinea pig, and human blood to direct feeding on hamsters using laboratory colonies of *Aedes aegypti* and four species of *Anopheles (dirus, cracens, minimus, sawadwongporni).* Feeding rates did not differ across blood types within species. However, survival, fecundity, and egg viability were lower in *Anopheles* species after feeding on sheep blood through an artificial membrane. Mortality rates in *An. sawadwongporni, An. cracens, An. dirus* and *An. minimus* engorged females are 52.3%, 48.8%, 14.8% and 14.3%, respectively. Mortality was significantly higher ($P<0.05$) using sheep blood fed compared to other blood types. Mortality was observed from 1 to 7 days post blood feed. *An. sawadwongporni* mortality was highest at day 1 (29.7%), *An. cracens* mortality peaked at day 3 (38.7%), while *An. minimus* and *An. dirus* mortality was much lower over all peaking at day 7 at 7.4%

and 5.7%, respectively..A decreasing rate of fecundity and hatching rate occurred as well. *An. dirus*, *An. cracens* and *An. sawadwongporni* produced significantly fewer eggs and showed lower hatching rates after feeding on sheep blood as compared to other blood sources. The findings support the conclusion that sheep blood is not a suitable protein source for maintenance of malaria vectors in Thailand.

## 245

### VARIATION IN CIBARIAL ARMATURE OF TWO MEMBERS OF THE *ANOPHELES GAMBIAE* S.L. EXHIBITING DIFFERENT CAPACITIES IN LYMPHATIC FILARIASIS TRANSMISSION

**Hilaria Amuzu**, Michael D. Wilson, Daniel A. Boakye

*Noguchi Memorial Institute for Medical Research, Legon, Accra, Ghana*

Elimination of lymphatic filariasis (LF) through mass drug administration (MDA) with ivermectin and albendazole in areas where *Anopheles* species transmit *Wuchereria bancrofti* is considered feasible due to the process of facilitation. The possible reason for facilitation is the presence of well developed cibarial armature which causes lethal abrasions to the parasites. Vectors with less developed or fewer number of cibarial armature are more efficient in the uptake of *W. bancrofti* at low level microfilariaemia. *Anopheles gambiae* s.l. in two endemic communities each with six rounds of MDA but differing transmission potentials were studied to determine the sibling species of *An. gambiae* s.l, molecular forms of *An. gambiae* s.s. and the stature and number of the cibarial armature. Mosquitoes were collected using the human landing method, identified morphologically and sibling species of *An. gambiae* s.l. and *An. gambiae* s.s. molecular forms determined by PCR and PCR-RFLP respectively. The length and width of the cibarial dome and cibarium were measured and the number of teeth counted for each mosquito identified and compared between sibling species and molecular forms. Only *An. gambiae* s.s was found in the community with lower ATP while *An. melas* and *An. gambiae* s.s were found at the site with high ATP. No significant differences were found in the length and width of the cibarium and cibarial dome of all species and forms. There was however a significant difference in the number of cibarial teeth (ANOVA F= 5.049, df=2, p=0.014). The mean number of teeth in *An. melas* was 14 (range= 12-15) which was significantly less than those of *An. gambiae* M form (mean=15.45; range=13-19) [ANOVA F=5.221, df=1, p= 0.032)] and S form (mean=16; range= 15-17) [ANOVA F=13.639, df= 1, p=0.002]. No significant difference (ANOVA F=0.471, df=1, p= 0.503) was found in the number of cibarial teeth of the M and S forms. This suggests that *An. melas* may exhibit limitation and hence where it occurs, MDA should be complimented with vector control to achieve LF elimination.

## 246

### BED NET COVERAGE, USAGE AND CONDITION IN FISHING VILLAGES OF SUBA DISTRICT, WESTERN KENYA

**Gabriel O. Dida**[1], George O. Sonye[2], H. Horio[3], S. Kaneko[3], M. Shimada[3], F. Kyoko[3], N. Minakawa[3]

*[1]School of Public Health, Maseno University, Kisumu, Kenya, [2]International Centre of Insect Physiology and Ecology, Mbita, Kenya, [3]Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan*

Past studies showed that bed nets can reduce child mortality. Currently, the Kenyan government is subsidizing the price of bed nets, and several non-governmental organizations (NGOs) are distributing nets with little charge. However, information on coverage, usage and condition of nets is lacking, particularly in the remote areas. We investigated bed net coverage and condition in seven fishing villages on the shore of Lake Victoria, Western Kenya. Usage of bed nets was examined through direct observation in early morning. As locals have started to replace traditional papyrus mats with bed nets for drying small fish, we also investigated how widely bed nets were used for capturing and drying fish. Coverage was 70.1% and 32.2% for mainland and island villages, respectively. We recognized 285 bed nets in early morning, and 178 (62.5%) of them were in use. However, 78 nets in use were not hanged properly. Sixty four nets were found in boxes, and the others were hanged, but not in use. We observed three types of nets; insecticide treated bet nets (13.4%), long-lasting treated bed nets (LLTNs, 49.8%), and untreated bed nets (37%). Nearly 90% of the observed nets had at least one hole more than 1cm. We found 234 bed nets being used for drying fish on beaches, and 194 (82.9%) of them were LLTNs. Forty one bed nets had been used for capturing fish. Locals preferred LLTNs for drying or capturing fish because they were stronger and fish dry faster and straighter. An NGO distributed 150 LLTNs in one village eight months before this survey, and we recognized 52 (36.7%) of them were being used for either drying or capturing fish. Over 95% of interviewed residents did not have any training on proper usage and maintenance of nets. In addition to pursuing high coverage of bed nets, an education component should be included in the ITN distribution.

## 247

### COMPARATIVE EVALUATION OF THE EFFECTIVENESS OF THE IFAKARA TENT TRAP, STANDARDIZED RESTING BOXES AND THE HUMAN LANDING CATCH FOR SAMPLING MALARIA VECTORS AND OTHER MOSQUITOES IN URBAN DAR ES SALAAM

**Maggy Sikulu**

*University of Nairobi, Nairobi, Kenya*

Frequent, sensitive and accurate sampling of *Anopheles* mosquitoes is a prerequisite for effective management of malaria vector control programs. The most reliable existing means to measure mosquito density is the human landing catch (HLC). However, the HLC technique raises major ethical concerns because of the necessity to expose humans to vectors of malaria and a variety of other pathogens. Furthermore, it is a very arduous undertaking that requires intense supervision which is severely limiting in terms of affordability, sustainability and scalability. A community-based, practical mosquito sampling protocol, using the Ifakara Tent Trap-B (ITTB) and standardized resting boxes (SRB), was developed and evaluated in terms of the number and sample composition of mosquitoes caught by each compared to rigorously controlled HLC. Mosquitoes were collected once and three times every week by the HLC and the alternative methods, respectively, in the same time and location. Overall, the three traps caught 44,848 mosquitoes. The ITT-B, HLC and SRB caught 168, 143 and 46 *An. gambiae* s.l. as well as 26315, 13258 and 4791 Culex species respectively. The ITT-B was 3 and 5 times cheaper than the HLC per mosquito caught for *An. gambiae* and Culex sp., respectively. Significant correlations between the numbers caught by HLC and ITT-B were observed for both *An. gambiae* s.l. ($P<0.001$) and Culex species ($P=0.003$). Correlation between the catches with HLC and SRB were observed for Culex species ($P<0.001$) but not *An. gambiae* s.l. ($P=0.195$), presumably because of the low density of the latter. Neither ITT-B nor SRB exhibited any obvious density dependence for sampling the two species. In conclusion, SRBs exhibited poor sensitivity for both mosquito taxa and are not recommended in this setting. However, this protocol is affordable and effective for routine use of the ITT-B under programmatic conditions. Nevertheless, we recommend that both the trap and protocol be evaluated further at full programmatic scales to establish effectiveness under fully representative conditions of routine practice.

## 248

### INTERMITTENT PREVENTIVE THERAPY (IPT) USE AMONG PREGNANT WOMEN ATTENDING ANTENATAL CLINICS IN PRIMARY HEALTH CARE CENTERS IN A RURAL LOCAL GOVERNMENT AREA IN NIGERIA

**Stella O. Akinleye**

*University of Ibadan, Ibadan, Oyo State, Nigeria, Ibadan, Nigeria*

Prevention of malaria in pregnancy is a major public health challenge. Despite the evidence of the effectiveness of sulphadoxine-pyrimethamine

(SP) in reducing the adverse effects of malaria during pregnancy when used for intermittent preventive therapy (IPT) the uptake and coverage of IPT during pregnancy in Nigeria is low. This study set out to assess the use of IPT among pregnant women attending primary health centres in rural area and determine factors responsible for the low uptake. A cross-sectional study of 209 pregnant women selected by systematic random sampling from among attendees of antenatal clinics in primary health care centres in a rural Local Government Area of Ekiti State, Nigeria was carried out. Information on knowledge of IPT, delivery, adherence and acceptability was obtained using an interviewer administered questionnaire. Descriptive statistics such as means, range, proportions were used and Chi-square test was used to examine association between categorical variables. All analyses were performed at 5% level of significance. One hundred and nine of 209 (52.2%) respondents have heard about IPT but only 26 (12.4%) were able to define it. Fifty seven (27.3%) reported to have received at least one dose of IPT during the index pregnancy and 21 of these (36.8%) took the SP in the clinic. Only three of the twenty-one (14.3%) were supervised by a health worker. Women who took their drugs outside of the clinic [22/36; 61.1%] would have liked to take their drugs in the clinic if allowed to bring their own cups probably due to hygiene. Almost half (25/57; 43.9%) of those who had used IPT during the index pregnancy expressed concern about possible adverse effect of SP on their pregnancies. Periodic shortages of SP in the clinics were reported. IPT use among pregnant women was very low in this study,there was poor adherence to the DOT scheme. More effort should be made to increase awareness on IPT. Health workers should be trained and monitored to ensure adherence.

## 249

### ECOLOGICAL CHARACTERIZATION OF *ANOPHELES* LARVAL HABITATS AND THEIR DISTRIBUTION IN WESTERN KENYA HIGHLANDS

**Bryson A. Ndenga**[1], Jemimah A. Simbauni[2], Jenard Mbugi[2], Andrew K. Githeko[1], Ulrike Fillinger[3]

*[1]Kenya Medical Research Institute, Kisumu, Kenya, [2]Kenyatta University, Nairobi, Kenya, [3]University of Durham, Durham, United Kingdom*

Understanding larval ecology of malaria vectors and the distribution of their breeding habitats within western Kenya highlands are highly needed in designing targeted larval control measures in space and time. Standard dipping and sweep net methods are used for larval sampling and mapped using Geographical Positioning System. Physical, chemical and biological parameters of selected aquatic habitats are performed. Production of malaria vectors from different aquatic habitat types and experimental pools is also being studied. A total of 1,129 aquatic habitats are sampled at Musilongo 28.4% (n = 431), Emutete 38.2% (n = 377) and Kezege 33.4% (n = 321). Out of these, Natural Swamps are 2.3% (n = 26), Cultivated Swamp 3.1% (n = 35), River Fringes 0.9% (n = 10), Puddles 4.6% (n = 52), Open Drains 73.5% (n = 830) and Burrow Pits 15.6% (n = 176). In a total of 7,089 times that all these habitats were sampled, 77.9% (n = 5,525) had water throughout and 38.4% (n = 2,122) had *Anopheles* early larval instars while 18.9% (n = 1,042) had late instars. Aquatic habitats with significantly ($p < 0.05$) higher *Anopheles* early and late instars larval densities have also higher habitat depths, algae coverage, vegetation heights, numbers of odonata nymphs and nitrate levels as opposed to those with significantly ($p < 0.05$) less *Anopheles* larvae, which have greater habitat widths, shade and scum coverage, higher *Culex* early instars larval densities and iron levels. Productivity of anopheline larva per $m^2$ varies among different habitat types with Natural Swamps having 1 larva/106.1$m^2$; Cultivated Swamps, 1/9.1$m^2$; River Fringes, 1/218.6$m^2$; Puddles, 1/0.5$m^2$; Open Drains, 1/6.9$m^2$ and Burrow Pits 1/13.0$m^2$. Experimental Pools sampled daily, 77.0% (n = 893) had water; 10,247 anopheline; 4,873 culicine larvae and 830 pupae counted. In conclusion, There is a possibility of identifying the most productive aquatic habitats for *Anopheles* mosquitoes and their distribution in space and time for the purpose of designing effective larval control measures.

## 250

### TRAPPING MALARIA VECTORS USING SYNTHETIC ODORS THAT ARE MORE ATTRACTIVE THAN HUMANS

**Fredros O. Okumu**

*Ifakara Health Institute, Ifakara, Morogoro region, United Republic of Tanzania*

Disease transmitting mosquitoes locate suitable hosts by deciphering their characteristic odor profiles. We developed a blend of synthetic human odorants and evaluated it as an attractant for the African malaria vector, *An. gambiae.* Field trials in rural Tanzania demonstrated that at long range, the blend attracts more mosquitoes than human beings, but that it is less attractive than humans at short range. These odor-blends can be readily developed into high impact interventions and accurate sampling tools for mosquitoes that transmit malaria and other diseases.

## 251

### TARGETING PRODUCTIVE CONTAINER AND POSITIVE HOUSES AS CONTROL STRATEGIES TO REDUCE *AEDES AEGYPTI* POPULATION DENSITY IN DENGUE ENDEMIC NEIGHBORHOODS

**Rafael Maciel-de-Freitas**, Mariana R. David, Anielly A. Ferreira

*Oswaldo Cruz Foundation, Rio de Janeiro, Brazil*

The main objective is to evaluate in dengue endemic neighborhoods of Rio de Janeiro the efficiency of two distinct control strategies to reduce *Aedes aegypti* population density: elimination of productive container types (Area1) and suppression of all container types in positive houses (Area2), comparing achieved results with a control site (Area3), where no intervention were produced. In the three study areas, control routine was unaltered. A one-year fieldwork started on July 2008 and consisted of adult collections with backpack aspirators and MosquiTRAPs in 40 randomly selected houses per week per area. Collected individuals had their wing sized and ovaries dissected to determine parity status and survival rate. Pupal surveys were done in intervals of 4 months (Set/08, Jan/09 and May/09) to determine container productivity and guidelines for interventions. In Area1 - where water tanks (WT) hold 72% of pupae - 733 of them were permanently covered with nylon net. Adult collection in the 5 subsequent weeks drastically reduced (2.8 times), but not lasted long. Surprisingly, 6 weeks later, adult collection returned to the level observed before intervention. In Jan/09, no pupae were collected in WT, but 47.8% of them were found in 74 metal drums (MD) (MD hold 3% of pupae in Set/08), which was also covered with nylon net. In Area2, before the first intervention, adult collection was similar as Area3. In Set/08 we eliminate all container types from 32 positive houses in Area2. In Jan/09, we found *Ae. aegypti* immatures in 38 houses, none of them was positive in Set/08. Since Set/08, adult collections in Area2 have been lower than in Area3, often even half of it. Overall, wing size decreased around 0.20mm after first intervention in Area1, but not in Area2, in comparison with Area3. In Area3, no significant changes on container productivity between pupal surveys were observed; around 30% of total pupae were collected in plant dishes. On 38$^{th}$ week, adult collection in Area1 remains as high as before interventions, contradicting the hypothesis that elimination of a productive container would result in a similar reduction in adult mosquito population density. Unexpectedly, these preliminary results suggest elimination of all container types from positive houses may be more efficient to reduce *Ae. aegypti* density than targeting the most productive container type in a given area.

## 252

### EFFECTS OF ENVIRONMENTAL VARIABLES AND PREDATORY ON SWARMING AND MATING BEHAVIOUR OF NATURAL POPULATIONS OF *ANOPHELES GAMBIAE* S.S. IN BURKINA FASO

**Simon P. Sawadogo**[1], Antoine Sanon[2], Idrissa Dicko[3], Moussa Namountougou[1], Hyacinthe K. Toe[1], Ali Ouari[4], Abdoulaye Diabate[1], Carlo Costantini[5], Jean-Bosco Ouedraogo[1], Robert T. Guiguemde[4], Roch K. Dabire[1]

*[1]Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso, Burkina Faso, [2]University of Ouagadougou, Ouagadougou, Burkina Faso, [3]Université polytechnique de Bobo Dioulasso, Bobo-Dioulasso, Burkina Faso, [4]Centre muraz, Bobo-Dioulasso, Burkina Faso, [5]IRD/OCEAC, Yaoundé, Cameroon*

The progress in genomic have increased the prospects of using genetically modified mosquitoes or sterilised males in malaria vector control. This strategy requires a proper understanding of potential interactions with naturally occurring populations. *Anopheles gambiae*, the main malaria vector in Africa, mates in swarms. Factors that may be involved in the swarming and mating system are poorly documented. We characterized swarming behaviour and determined whether predatory presence affect mating behaviour of *An. gambiae*. Swarms of *An. gambiae* s.s. were followed up from July 2006 to December 2008 in Vallée du Kou and Soumousso situated in southwestern Burkina Faso. Environmental parameters such as light intensity, temperature, and relative humidity were recorded at the time of swarm formation. We determined the number of mating and predation intrusion. A copulating were noted when a pair of mosquitoes was seen leaving a swarm. A predation event was noted when a predator swooped into a swarm, slowed down and dipped into the mass of mosquitoes, and flew out. We investigated the predatory intrusion following the dynamic of mating. The first indicator of swarming occurrence in the rice area was the apparition of dragonflies. Before swarming occurred, one or two dragonflies started flying insistently around the swarming place at the same height. After five minutes the first male pointed up flying in zigzag movement. It was rejoined by other males. Males swarmed 0.6-4 m above field markers constituted by stored wood, wells, heaps of refuse and open areas. The environmental parameters at swarm starting and ending showed substantial spatio-temporal variation. We observed that mating was not exponential but varied following the intrusion of dragonflies. This study showed that the environmental parameters we measured were not correlated with the swarm formation, and that Predatory intrusion perturbed mating and swarming behaviour.

## 253

### SEASONAL ABUNDANCE AND NATURAL INFECTION OF THE SAND FLY *LUTZOMYIA LONGIFLOCOSA* IN A DOMESTIC FOCUS OF AMERICAN CUTANEOUS LEISHMANIASIS IN CHAPARRAL, TOLIMA, COLOMBIA

**Dairo Marin**[1], Cristina Ferro[1], Leonard Munstermann[2], Clara Ocampo[1]

*[1]Centro Internacional de Entrenamiento e Investigaciones Medicas, CIDEIM, Cali, Colombia, [2]Yale University School of Public Health, New Haven, CT, United States*

The sand fly *Lutzomyia longiflocosa,* is considered the principal vector of cutaneous leishmaniasis (CL) in the Sub Andean region of Colombia. In 2004, this was the most abundant species found in the municipality of Chaparral during the largest CL outbreak ever recorded in the country. The main aim of this work was to identify temporal abundance patterns of *L. longiflocosa* during high and low precipitation periods, and natural infection by *Leishmania* in different environments in the rural township of Agua Bonita in Chaparral. This township registered high cumulative incidence during the CL outbreak. CDC light traps were set up monthly in 3 houses for 3 consecutive nights for 11 months beginning in June 2007. The three houses were selected on the basis of previous observation of high indoors sand fly abundance. Five traps were set per house: one indoors, 2 peridomestically (10 m away from house) and 2 in neighboring woodland. Sand fly abundance data was linked to local climatic data from IDEAM (National agency). Natural infection was determined in pools (10<n>20) using PCR-Southern-Blot with kDNA as a target. The months with lower precipitation, August and February, presented the highest sandfly capture (28.6% and 31.8% of 9,420 collected respectively). *L. longiflocosa* was the most abundant species collected every month in all environments and captures were higher in woodland environments (44.14% per trap per night) and peridomestic (44.08%) than indoors (11.8%). Southern-Blot revealed amplification in 10pools out of 33 indoor environments (n=451, 2.2%), followed by peridomestic (14p/138; n=2.498, 0.6%) and woodlands (3p/134, n= 2.490, 0.1%). Verification of the PCR product by DNA sequencing is ongoing. The presence of *L. longiflocosa* and the high prevalence of infection observed indoors and in the peridomestic environment suggest that the transmission is associated with the house. These findings explain the high proportion of women and children infected during outbreaks and suggest the presence of a reservoir associated with the peridomestic environment.

## 254

### URBAN STREAM POLLUTION INCREASES MOSQUITO FITNESS AND DISEASE VECTOR POTENTIAL

Carolyn L. Keogh, Uriel D. Kitron, Andy M. Nguyen, Gonzalo Vazquez-Prokopec, **Luis F. Chaves**

*Emory University, Atlanta, GA, United States*

Anthropogenic disturbance of natural cycles in organic elements (Nitrogen and Phosphorus) is one of the major causes of the disruption of species interactions across ecosystems. In urban landscapes the crowding of humans and their waste products in freshwater systems is likely to exacerbate distressing patterns of intra and inter-specific interactions, especially those relevant for disease emergence. Here, we present the results of field observations and two semi-natural experiments addressing the effects of ecosystem level changes on the density dependent fitness of the most common tropical and subtropical urban mosquito, Culex quinquefasciatus Say (Diptera: Culicidae). The semi-natural experiments were designed to quantify both the oviposition preference for water influenced by sewage overflows, and the relative importance of density, weather variability and water quality on larval mortality, sex ratio and size at adult emergence. These phenotypic traits are important in determining vectorial capacity for the transmission of several pathogens.The field observations showed this mosquito species to be present in streams where N and P levels were significantly higher than in streams where it was absent, the difference mediated by the effects of combined sewage overflows. The oviposition experiment showed water from these systems to be more attractive for oviposition by this mosquito species than water uninfluenced by the sewage overflows. The density-dependence experiment revealed that mortality hazards were independent of larval density, decreased in sewage overflow water and increased with raising minimum temperatures. Under all rearing conditions adult mosquito size decreased with density. Mosquitoes from sewage overflow water emerged faster, were bigger and had an increased ratio of females to males. All these traits could determine the density dependent regulation of mosquito populations and the ability to transmit pathogens through size mediated fecundity and mating induced feeding behavior. Finally, our results show the importance of urban stream quality as a factor for the urban emergence of arboviral diseases, calling for the implementation of environmentally sound strategies for water management, given the potential to diminish the risk of some vector-borne diseases and other health hazards, while conserving biodiversity in cities.

## 255

### BIOLOGICAL DIVERSITY AND GENE POLYMORPHISMS ASSOCIATED WITH KNOCKDOWN RESISTANCE IN MEMBERS OF THE PAPUA NEW GUINEA *ANOPHELES PUNCTULATUS* SPECIES COMPLEX

**Cara N. Henry-Halldin**[1], Kogulan Nadesakumaran[1], Daphne Sepe[2], John B. Keven[2], Laurie Gray[1], Lisa Reimer[1], Nigel W. Beebe[3], Robert D. Cooper[4], Peter A. Zimmerman[1]

*[1]Case Western Reserve University, Cleveland, OH, United States, [2]Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea, [3]School of Integrative Biology, University of Queensland, St. Lucia, Australia, [4]Australian Army Malaria Institute, Enoggera, Australia*

Limited work has been done to define diversity among the *Anopheles punctulatus* species complex mosquitoes (*A. punctulatus*[*Ap*], morphologically indistinguishable *A. farauti* 1-8[*Af*], *A. koliensis*[*Ak*]) in Papua New Guinea (PNG). Evaluation of marker genes is necessary to understand biodiversity of PNG Anophelines. The *kdr* mutation in the voltage gated sodium channel gene (VGSC), conferring knockdown resistance to pyrethroids and DDT, provides an additional genetic marker of interest given its potential association with pyrethroid resistance. To characterize the biodiversity of *Ap* complex we have analyzed 13 isolates, collected from the Madang and East Sepik Provinces in PNG, for genetic markers including portions of the internal transcribed spacer 2 rDNA(ITS2), cytochrome oxidase I(COI), 18SrDNA genes and the VGSC gene fragment containing *kdr*. Sequence alignment and phylogenetic analysis was performed using ClustalW and NJ method. Analysis included previously published sequences and used African *Anopheles* species, *A.gambiae*(*Ag*) and *A. funestus*(*Afn*), as outgroups. Single nucleotide polymorphisms (SNPs) were observed among members of different species. Some SNPs, however, were only found within members of the same species. In contrast to comparisons between *Ap* complex and *Ag* and *Afn* (Alignment score 55-66; *Ag* vs *Afn*=90) the alignment scores for 18SrDNA (723-836bp) among members of the *Ap* complex were more similar (*Ap* vs *Ak* = 90-91; *Ap* vs *Af1*=87-91; *Ap* vs *Af4*=93-94; *Ak* vs *Af1* =96; *Af1* vs *Af4*=93-94). Observed alignment scores for 18SrDNA comparison within species from previously published sequences and our present collections were *Ap*=94-97 (distance between collection sites: 140km), *Af4*=99 (440km), *Af1*=99-100 (720km), *Ak*=99 (1600km). Individual *Af1* and *Ak* samples were separated by the Bismarck and Solomon Seas respectfully. Understanding the genetic diversity of malaria vectors in endemic regions becomes a necessary part of malaria control and integrated vector management. Since long lasting insecticide-treated bednets (LLINs) are being distributed throughout PNG, it is important to evaluate genetic markers like those chosen here to monitor mosquito-targeting control measures. These tools will enable evaluation of overall genetic diversity within and between vector populations (species and strains) and elucidate associations between specific mutations (*kdr*) and susceptibility to pyrethroids used in LLINs.

## 256

### XENOMONITORING FOR ZOONOTIC FILARIASIS IN NORTH DAKOTA

**Joseph O. Mehus**, Jeffery A. Bell, Jefferson A. Vaughan

*University of North Dakota, Grand Forks, ND, United States*

Filarial parasites use various vertebrate animals across North Dakota as hosts. While harvesting and trapping animals for necropsy or taking blood samples has been the traditional procedure for identifying infected host animals, it is often labor intensive and adult parasites are difficult to find. As an alternative, xenomonitoring capitalizes on the fact that mosquitoes naturally feed on vertebrate hosts and ingest the microfilaria. Thus, it may be easier to sample the mosquito population in an area rather than sampling the vertebrate communities. We first wanted to know how long microfilarial DNA persisted in blood-fed mosquitoes. *Culex pipiens* were fed on a microfilaremic grackle and sampled over a 10 day period. For each sample, DNA was extracted and PCR was performed using primers designed to amplify the 18s rRNA gene of the avian filarid, Chandlerella quiscali. Microfilarial DNA was detectable up to 10 days post feeding. During the summer of 2008, we collected mosquitoes in rural eastern North Dakota using Mosquito Magnet traps. Sixty-six mosquitoes contained blood meals and the DNA was extracted from these engorged mosquitoes. Of the 14 mosquitoes tested by our filarid-specific PCR assay thus far, 10 (71%) were PCR positive for filarid DNA. The high percentage of filarid-positive bloodmeals suggests that the prevalence of filarial infections in the local wild life within this area is high. The parasitological literature indicates that the prevalence of filarial infections in wild birds and mammals can be very high, often exceeding 70%. Thus, our preliminary xenomonitoring results are not inconsistent with what is known using more traditional methods of determining zoonotic filarial infection rates. Future studies will include sequencing and identification of the microfilariae, as well as sequencing and identification of the vertebrate host. In this way, xenomonitoring may also be used to help establish host-parasite species relationships.

## 257

### INTERACTIONS BETWEEN BACTERIA ASSOCIATED WITH MOSQUITO DIGESTIVE TRACTS AND LA CROSSE VIRUS

**Justin R. Anderson**, Jonathan D. Joyce, Amber A. Bales

*Radford University, Radford, VA, United States*

Arthropod-borne viruses continue to be important causes of global morbidity and mortality, despite continued efforts to intervene with transmission. La Crosse virus (LACV) is an etiologic agent of pediatric encephalitis in the eastern United States, and serves as a model for other arboviruses. Several competent LACV vectors are present in western Virginia, including the natural vector *Ochlerotatus triseriatus* and the exotic species *Aedes albopictus* and *Ochlerotatus japonicus*. Host-seeking females of these species were collected and the midguts and diverticula were dissected into sterile saline. Culturable aerobic bacteria were grown on nutrient agar, and individual colonies were isolated in pure culture and identified by sequencing the 16S ribosomal RNA gene. These bacteria were also tested for direct interactions with LACV using a plaque-reducing assay in Vero cells. Bacteria from the order *Lactobacillales* and the following families were isolated: *Acetobacteriaceae, Bacillaceae, Enterobacteriaceae, Enterococcaceae, Flavobacteriaceae, Microbacteriaceae, Paenibacillaceae, Pseudomonadaceae, Sphingobacteriaceae, Sphingomonadaceae*. Approximately 50% of these isolates reduced virus infectivity of Vero cells by at least 30%. These bacteria represent attractive candidates for selection of increased LACV binding or for paratransgenic engineering to prevent LACV transmission by vector mosquitoes.

## 258

### DYNAMICS OF MALARIA PREVALENCE AND VECTOR ABUNDANCE IN SENTINEL SITES IN WESTERN KENYA: ROLE OF INSECTICIDE-TREATED BEDNETS AND ARTEMISININ-BASED COMBINATION THERAPY

**Guofa Zhou**[1], Yaw Afrane[2], Andrew K. Githeko[3], Guiyun Yan[1]

*[1]University of California at Irvine, Irvine, CA, United States, [2]International Center of Insect Physiology and Ecology, Nairobi, Kenya, [3]Kenya Medical Research Institute, Kisumu, Kenya*

Major malaria control programs have been launched in Africa in the past several years with the support of the Global Fund, the President's Malaria Initiative and private foundations. The ongoing malaria control programs have largely focused on increasing insecticide-treated bed net (ITN) coverage and artemisinin-based combination therapy (ACT) drug delivery. However, most programs lack critical monitoring and evaluations of the epidemiological and entomological impacts of the malaria control measures. The objective of this study is to examine the impact of the malaria control measures on malaria prevalence and malaria transmission,

using two sentinel sites in malaria-epidemic highland and one site in endemic lowland in western Kenya. Through monthly field survey, we determined the dynamics of malaria prevalence and vector abundance from 2002-2009. Using questionnaire survey, we determined the use of ITN and antimalarial drug use practices. We found that malaria prevalence remained high and stable between 2002 and June 2006 in all three sites, and then a dramatic decline during the second half of 2006 (80-90% reduction in comparison to the first half of 2006). Abundance of malaria vectors were high in all three places from 2002-2005, but fell by about 90% since 2006 in all three sites. From 2007 on, malaria prevalence remained low in the two highland sites, but rebounded in the lowland site. ITN coverage was gradually increased from about 10% in 2002 to 70% in 2009 in the three sites. The sharp reduction in malaria prevalence in the second half of 2006 was coincidental with the large-scale distribution of ACT in Kenya during that particular period of time and dramatic reduction in vector abundance, probably as a result of introduction of new long-lasting ITN and abnormal weather conditions. The recovery of malaria prevalence in the lowland site since 2007 was likely associated with the inadequate supply of ACT, and the use of quinine, amodiaquine and sulfadoxine-pyrimethamine (SP) drugs. We are currently conducting a case-control study to determine the role of vector abundance, antimalarial drug use and drug resistance on clinical malaria.

## 259

### NOSOCOMIAL TRANSMISSION OF PULMONARY TUBERCULOSIS IN A SECONDARY HEALTH-CARE FACILITY IN NIGERIA

**Aderemi O. Kehinde**[1], Abubakar Baba[2], Rasheed A. Bakare[1], Olusoji M. Ige[1], Confort Gbadeyanka[3]

*[1]College of Medicine, University of Ibadan, University College Hospital, Ibadan, Nigeria, [2]University College Hospital, Ibadan, Nigeria, [3]Jericho Chest Hospital, Ibadan, Nigeria*

Tuberculosis (TB) infection control remains a public health priority especially now with the emergence of extensively drug resistant TB. TB infection control programs are not routinely implemented in many endemic sub-Saharan Africa countries which harbor significant burden of TB globally. In Nigeria, however, primary focus is mainly on implementation of DOTS (Directly Observed Treatment Short Course). The study was carried out to determine the prevalence of hospital acquired TB among health care workers (HCWs) at two designated DOTS centers in Ibadan, Nigeria. From January - June 2008, all consenting HCWs at the DOTS centers located within University College Hospital (UCH) and Jericho Chest Hospital, Ibadan, Nigeria were screened for pulmonary TB. Socio-demographic data of the subjects were obtained by administering standardized questionnaire Three early morning sputa were collected from each subject. The sputa were processed at the TB laboratory of the Department of Medical Microbiology, UCH, Ibadan, Nigeria. Each sputum was stained for acid fast- bacilli (AFB) with Zeihl-Neelsen reagents. A known AFB stained slide and a slide stained with egg albumin were used as positive and negative controls. Results were read according to grading system of the International Union Against TB and Lung Diseases. The sputum was then inoculated onto prepared acid buffered Ogawa medium and incubated at 37$^{o}$C for six weeks. *M. tuberculosis* strain H37RV and sterile Ogawa medium were used as positive and negative controls respectively. A total of 271 subjects were studied. Five (1.8%) were physicians, 36 (13.3%) were nurses while laboratory scientists, laboratory assistants and hospital maids accounted for 12 (4.4%), four (1.5%) and three (1.1%) respectively. The majority 211 (77.9%) were students. All those with positive results were from Jericho chest clinic, a secondary health care facility. Nine subjects (3.3%) were positive for AFB while five (1.8%) had their sputum positive on culture. Of those with positive AFB, seven were from students (77.8%), one (1.2%) each from hospital maid and laboratory assistant cadres. Contamination rate was found to be 3.3%. In conclusion, from the study, hospital acquired TB is more prevalent at the secondary health care facility. This calls for implementation of TB infection control strategies in all health care facilities including the community health centers.

## 260

### TUBERCULOSIS DIAGNOSTIC DELAY IN HIGH-INCIDENCE SHANTYTOWN COMMUNITIES

**Samuel G. Schumacher**[1], Rosario Montoya[2], Robert H. Gilman[3], Jessica Franco[2], Betty Valiente[2], Silvia Carrera[2], Karine Zevallos[2], Dami Onifade[2], Colleen Acosta[4], Carlton A. Evans[5]

*[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Asociación Benefica Prisma, Lima, Peru, [3]Johns Hopkins Bloomberg School of Hygiene and Public Health, Baltimore, MD, United States, [4]IFHAD: Innovation For Health And Development, London, United Kingdom, [5]London School of Hygiene & Tropical Medicine, London, United Kingdom*

Delayed tuberculosis (TB) diagnosis increases morbidity, mortality and the risk of TB transmission. Sputum microscopy is the principal TB diagnostic test globally but is insensitive, so patients often require repeated testing. We therefore studied the relative contribution of health-seeking behavior and suboptimal tests to the total delay in TB diagnosis. Newly diagnosed TB patients (n=233) in 16 Peruvian shantytowns were interviewed to determine their 'health-seeking delay' from symptom onset until visiting a medical facility and their subsequent 'testing-delay' until their TB was diagnosed, principally by sputum microscopy. The health-seeking delay was considerably greater than the testing-delay (median 21 vs 2 days) and patients started their treatment soon after being diagnosed (median 1 day). Diagnostic delays were characterized in 3 distinct groups: (1) Rapid Diagnosis. Most patients (60%) had a relatively short total diagnostic delay from symptom onset to diagnosis (median 21 days, IQR 10-25; maximum 35). These patients sought medical care within a median of two weeks and were diagnosed a median of one day later; (2) Delayed Health Seeking. A further 27% of patients had prolonged symptoms before visiting a health-post (median 70 days, IQR 59-115; maximum 365) but were then diagnosed rapidly (median 1 day; IQR 1-10; maximum 30); and (3) Prolonged Testing. The remaining 13% of patients visited a health post soon after symptom onset (median 15 days; IQR 10-30; maximum 45) but then had a prolonged testing delay (median 50 days, IQR 38-76; maximum 150). In conclusion, most patients were diagnosed soon after symptom onset. Delayed health-seeking made a much greater contribution to total diagnostic delay than the subsequent time required for tests to make the diagnosis. Increasing test speed and sensitivity should accelerate TB diagnosis, but there is greater potential impact from interventions that encourage patients to seek medical care earlier in their illness.

## 261

### TUBERCULOSIS AND NUTRITIONAL STATUS IN TRANSITIONAL COMMUNITIES

**Chulanee Jongkaewwattana**[1], Samuel G. Schumacher[1], Karine Zevallos[2], Mathew Baldwin[2], Alejandro Necochea[2], Rosario Montoya[2], Robert H. Gilman[3], Carlton A. Evans[4]

*[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Asociación Benefica Prisma, Lima, Peru, [3]Johns Hopkins Bloomberg School of Hygiene and Public Health, Baltimore, MD, United States, [4]Wellcome Centre for Clinical Tropical Medicine Imperial College, London, United Kingdom*

Tuberculosis (TB) is associated with wasting and was traditionally called 'consumption'. However, little is known about the interaction between TB and the epidemic of obesity in transitional societies, where TB is particularly common. We, therefore, studied the association between anthropometric measures and TB disease in transitional shantytown communities with high TB incidence. 3,123 adult (>17 years) participants were recruited continuously over a six-year period in 16 adjacent Peruvian shantytowns where the average daily income was <$1US/day. Weight and height were measured in three cohorts: TB patients (n=898), 'TB-suspects' who had symptoms suggestive of TB but whose TB tests were negative (n=83) and healthy controls (n=2,142). TB patients had lower

body mass index (BMI) than TB-suspects who had lower BMI than controls (both p<0.001). Mean (standard deviation) BMI was 21.8 (3.1) kg/m$^2$ for patients, 24.1 (4.6) kg/m$^2$ for TB-suspects and 25.7 (4.4) kg/m$^2$ for controls. However, 16% of patients were overweight (BMI>25 kg/m$^2$) and only 29% of TB patients were underweight (<20 kg/m$^2$). In contrast, 67% of controls were overweight (p<0.0001) and only 9% of controls were underweight (P<0.0001). There was a strong association between increasing height and TB disease (p<0.001). Mean (standard deviation) height was 158 (8.8) cm for patients, 157 (9.2) cm for TB-suspects and 156 (8.5) cm for controls. In multiple regression analysis adjusting for age (odds ratio [OR] 1.002 95% confidence intervals [CI] 0.99-1.01), male gender (OR 2.06 95%CI 1.61-2.63) and weight (OR 0.89 95%CI 0.88-0.90), increased height was associated with significantly greater odds of TB disease (p<0.001; odds ratio 1.07 95%CI 1.05-1.09 per cm increase in height). In conclusion, **i**n these transitional peri-urban shantytowns, there was a strong independent association between increased height and TB disease. A significant minority of TB patients were overweight, despite the overall association between low bodyweight and TB.

## 262

### PREVALENCE OF FLUOROQUINOLONE RESISTANCE AMONG TUBERCULOSIS PATIENTS IN SHANGHAI, CHINA

**Xia Li**[1], Peng Xu[2], Qian Gao[1]

*[1]Fudan University, Shanghai, China, [2]Shanghai Centers for Disease Control and Prevention, Shanghai, China*

Fluoroquinolones are useful antibiotics against tuberculosis (TB). However, the prevalence level of fluoroquinolone resistance among TB patients is unknown, and the factors associated with fluoroquinolone resistance have yet to be determined. We performed a case-control study to identify the covariates that were associated with fluoroquinolone resistance among 605 clinical TB patients in Shanghai, China. Mutations in the quinolone resistance determining region of *gyrA* were good molecular markers of the fluoroquinolone resistance phenotype in Shanghai. Using this method, 1.9% of the clinical isolates that were pan-susceptible to first-line anti-TB drugs and 25.1% of the MDR isolates had a mutation in *gyrA*. By multivariate analysis, *gyrA* mutations were independently associated with MDR strains of *M. tuberculosis* (adjusted odds ratio (AOR) = 13.8), mono-resistance to rifampin (AOR = 6.3), poly-resistant strains (AOR = 4.5), age ≥ 46 years (AOR = 2.4) and retreatment cases of TB (AOR = 2.1). The prevalence of fluoroquinolone resistance was low among clinical isolates that were pan-susceptible to first-line anti-TB drugs. However, fluoroquinolone resistance was associated with resistance to first-line drugs and prior tuberculosis treatment. Acquired fluoroqinolone resistance might be prevented by appropriate treatment regimens for MDR TB patients, directly observed therapy and careful monitoring of anti-TB therapy.

## 263

### AN EFFECTIVE INFLUENZA VACCINE CANDIDATE BASED ON BACULOVIRUS PSEUDOTYPING

**Xianchun Tang**, Ted Ross

*University of Pittsburgh, Pittsburgh, PA, United States*

Hemagglutinin (HA) is the primary immunogen on the envelope of influenza virus. gp64 is the major envelope protein of baculovirus. Both HA and gp64 are type I membrane protein and exist in trimeric forms. Previous studies reported that HA-pseudotyped baculovirus could elicit hemagglutination inhibition (HAI) titers in the mouse model and demonstrated the potential of HA-pseudotyped baculovirus as an influenza vaccine. In order to investigate the factors that influence HA pseudotyping, we generated six chimeric gene constructs in which the coding sequences for the signal peptide (SP), transmembrane (TM) and cytoplasmic tail (CT) domains of HA were replaced with those of gp64: Bac-HA, Bac-spHA, Bac-spHAct, Bac-HAct, Bac-HAtmct, Bac-spHAtmct. All constructs express HA in Sf-9 cells and the chimeric HAs are pseudotyped on the surfaces of the recombinant baculovirus and maintained their hemagglutination activity. When tested at identical virus titers, the Bac-spHAct exhibited the highest HA titer. These data imply that the SP and CT of gp64 enhance HA incorporation into baculovirus, but TM impairs HA incorporation. Mice received 2 immunizations intranasally or intramuscularly, then challenged with a lethal dose PR8 virus (500pfus). All mice received Bac-HA immunization induced high HAI titer and survived without any weight loss after challenging. Mice that received recombinant HA survived with ~10% weight loss. However, all mice that received live *wt* baculovirus intramuscularly, BPL-inactivated *wt* baculovirus intranasally or mock vaccinated, died. Our results indicated that HA-pseudotyped baculovirus was able to confer 100% protection, which was better than recombinant HA. Moreover, since baculovirus-based vaccine candidates exhibit strong adjuvant properties due to the inherent properties of the baculovirus backbone, it may explain the strong immunogenicity of our vaccine candidates. Taken together, our data suggest that HA-pseudotyped baculovirus can be a promising strategy for the development of a safe and effective vaccine to control the spread of influenza virus.

## 264

### ANALYSIS OF STRAIN TRANSMISSION DURING AN EPIDEMIC OF MULTI-DRUG RESISTANT TUBERCULOSIS AMONG AIDS PATIENTS RECEIVING DIRECTLY OBSERVED THERAPY SHORT-COURSE (DOTS)

**Maria-Graciela Hollm-Delgado**[1], Fanny Arenas[2], Juliana Cordova[2], Patricia Sheen[2], Carlton A. Evans[3], Eduardo Ticona[4], Robert H. Gilman on behalf of TB Collaborative Group[5]

*[1]Université de Montréal, Montréal, QC, Canada, [2]Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Imperial College London, London, United Kingdom, [4]Hospital Dos de Mayo, Lima, Peru, [5]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States*

Infection with HIV has been associated with an increased risk of tuberculosis, but the link between HIV/AIDS and the development of drug-resistant tuberculosis remains unclear. This study examined the role of AIDS on strain transmission during an epidemic of multi-drug resistant tuberculosis (MDR-TB) among patients receiving Directly Observed Therapy Short-Course (DOTS). We identified two patient populations receiving DOTS for pulmonary tuberculosis in Lima, Peru between 1999 and 2005, based on group prevalence of AIDS [n=205 in *AIDS* cohort; n=386 in *non-AIDS* cohort]. All patients had physical exams and were interviewed before starting treatment. Sputum samples were collected at study enrolment, as well as week 1, months 1, 2, 4 and at the end of DOTS. DNA fingerprints for MDR-TB samples were ascertained using spoligotype and 1S6110 Restriction Fragment Length Polymorphism. Our analysis revealed that AIDS cohort patients were at greater risk of acquiring multi-drug resistance during DOTS as compared to those in the non-AIDS cohort [Incidence Rate Ratio: 2.14, 90% CI: 1.0 to 4.5]. While 92% (11/12) of AIDS cohort patients who developed multi-drug resistance were infected by a different strain of *M. tuberculosis* than at the start of DOTS, we found that the proportion of DOTS patients who developed multi-drug resistance by a different strain did not differ between cohorts (Fisher's Exact Test p=1.00). AIDS cohort patients who acquired MDR-TB were more likely to be infected by one strain type of *M. tuberculosis* (Fisher's Exact Test p=0.04). However, risk of super-infection with this strain did not differ by AIDS status after adjusting for patients' baseline risk of exposure to circulating strains [Standardized Incidence Ratio for AIDS cohort: 1.63, 95% CI: 0.5 to 3.8 and for non-AIDS cohorts 1.75, 95% CI: 0.4 to 5.1]. In conclusion, acquisition of multi-drug resistance during DOTS was often due to a different infection with MDR-TB rather than the development of drug-resistance within an existing strain. Although most AIDS-cohort patients who acquired MDR-TB during DOTS became infected by one particular strain of *M. tuberculosis*, this may have been a consequence of the strain's higher prevalence among HIV-infected patients at large.

## 265

### EXAMINING THE ENVIRONMENTAL EFFECTS ON INFLUENZA TRANSMISSION IN WARM CLIMATE USING NEURAL NETWORK

**Radina P. Soebiyanto**, Farida Adimi, Richard Kiang

*NASA Goddard Space Flight Center, Greenbelt, MD, United States*

Each year influenza epidemics causes up to 5 million severe illnesses and 500,000 deaths worldwide. Despite annual epidemics, influenza seasonality remains poorly understood. Prevailing theories on the seasonality vary from social contact behavior (school closing, air travel, etc.) to environmental factors. In temperate regions, influenza peaking in winter can be explained by the low temperature and humidity that can increase transmission by - among others - enhancing virus survivability outside the body, and promoting indoor crowding tendency. By contrast in some regions with warmer climate, influenza can peak twice a year. The role of environmental factors is arguable, due to the geographic variation of associated factors, and the elusive mechanism to increase transmission. In order to assess the environmental parameters and influenza seasonality in warmer climate, we analyze influenza incidences in Hong Kong. The climate in Hong Kong is considered to be tropical with temperature range of 14°C - 31°C, and an influenza pattern that typically peaks in spring and summer. We further compare the seasonality pattern in Hong Kong to that in Arizona - specifically in Maricopa County - since it has a similar temperature range but it exhibits influenza patterns similar to those in temperate regions (distinct peak in the winter). The environmental parameters and influenza incidences were modeled using a neural network in order to capture the unknown nonlinear relationship. The availability of remotely-sensed environmental data from NASA satellites enables us to develop a tool for predicting influenza cases which in turn can help in reducing the burden of annual influenza epidemics. Furthermore, identification of influenza-associated environmental parameters could enhance further biological studies on the transmission mechanism.

## 266

### A RARE CASE OF NECROTIZING GRANULOMATOUS PLEURITIS PRESENTING WITH EMPYEMA

**Javeria Shakil**, Deborah Asnis

*Flushing Hospital Medical Center, Flushing, NY, United States*

Tuberculous pleural effusion accounts for approximately 5% of all disease due to *Mycobacterium tuberculosis* (MTB) and is the second most common form of extrapulmonary tuberculosis (TB) after lymphatic involvement. Tuberculous pleural effusions are either a self limited reactive or much less commonly tuberculous empyema. We present a 30 year old patient with paucity of symptoms who was diagnosed with Tuberculous empyema. Case: A 30 year old male immigrant from Philippines presented with low grade intermittent fever and generalized myalgia for 1 week. Physical examination revealed low grade fever, no lymphadenopathy, reduced air entry in left chest with dullness and egophony. CT scan revealed dense pleural based consolidation of left upper lobe, small nodules in lower lobes, calcified pleural thickening and loculated left pleural effusion. His multiple sputum smears were negative for acid fast bacilli (AFB). He had positive Tuberculin skin test but negative HIV test. Thoracentesis showed Lymphocytes predominant exudate. He underwent left thoracotomy for decortication of thick pleural peel and drainage of empyema. Pleural biopsy showed necrotizing granulomatous pleuritis, rare AFBs and negative fungal stains. Cultures and PCR tests confirmed the diagnosis of tuberculous empyema due to MTB. Tuberculous empyema represents a chronic, active infection of the pleural space. Empyema is rare compared to reactive effusions in TB. It results from numerous organisms spilling into pleural space usually from rupture of a cavity, or via a bronchopleural fistula. Trapped lung causes the inability to re-expand and difficulty in achieving therapeutic drug levels in pleural fluid leading to drug resistance. Pleural fluid is predominantly lymphocytic exudate devoid of mesothelial cells. Pleural fluid smears are rarely positive but fluid cultures are positive in 25%. In contrast, thoracoscopic pleural biopsy and culture is positive more than 80% of the time. In conclusion, less common than Tuberculous pleurisy, tuberculous empyema represents a chronic active infection of the pleural space. It can run its course for decades, with a surprising paucity of clinical symptoms. In general, therapy is surgical, with a wide range of possible interventions including Parietal decortication, thoracoplasty, with or without omentopexy/myoplasty. Medical therapy is mandatory in an attempt to sterilize all residual TB foci.

## 267

### MOLECULAR CHARACTERIZATION OF ADENOVIRUS CIRCULATING IN CENTRAL AND SOUTH AMERICA DURING THE 2006-2008 PERIOD

**Josefina Garcia**[1], Merly Sovero[1], V. Alberto Laguna-Torres[1], Jorge Gomez[2], Wilson Chicaiza[3], Melvin Barrantes[4], Felix Sanchez[5], Mirna Jimenez[6], Guillermo Comach[7], Ivette De Rivera[8], Roberto Agudo[9], Ana E. Arango[10], Alma Barboza[11], Nicolas Aguayo[11], Tadeusz Kochel[1]

*[1]Naval Medical Research Center Detachment, Lima, Peru, [2]Direccion General de Epidemiologia, Lima, Peru, [3]Hospital Vozandes, Quito, Ecuador, [4]Hospital San Francisco Solano, Buenos Aires, Argentina, [5]Hospital Infantil Manuel de Jesus Rivera, Managua, Nicaragua, [6]Hospital Nacional Metapan, Santa Ana, El Salvador, [7]Laboratorio Regional de Diagnostico e Investigacion del Dengue y otras Enfermedades Virales, Maracay, Venezuela, [8]Universidad Nacional Autonoma de Honduras, Tegucigalpa, Honduras, [9]Servicio Departamental de Salud, Cochabamba, Bolivia, [10]Grupo de Inmunovirologia, Universidad de Antioquia, Medellin, Colombia, [11]Asociacion Rayos de Sol, Asuncion, Paraguay*

Human Adenoviruses (HAdvs) are well recognized pathogens, causing a broad spectrum of diseases. Serotype identification is critical for epidemiological surveillance, detection of new strains and understanding of HAdv pathogenesis. Little data is available about HAdvs subtypes circulating in Latin America. In this study, we have molecularly characterized 213 adenovirus collected from influenza like illness (ILI) presenting patients, during the 2006-2008 period, in 9 countries from Central and South America. Viruses, isolated from symptomatic ILI patients throughout the region were isolated by cell culture, amplified and sequenced. Our results indicate that 161(76%) adenoviruses belong to subgroup C, 45 (21%) to subgroup B and 7 (3%) to subtype E4. In conclusion, surveillance of human illnesses is the key to understanding the current distribution of viruses, and this knowledge is necessary to improve public health.

## 268

### RESISTANCE TO NEURAMINIDASE INHIBITORS IN INFLUENZA A/H1N1 IN LATIN AMERICA: EUROPEAN STRAINS ARRIVE TO THE NEW CONTINENT

**Josefina Garcia**[1], Merly Sovero[1], V. Alberto Laguna-Torres[1], Jorge Gomez[2], Wilson Chicaiza[3], Melvin Barrantes[4], Felix Sanchez[5], Mirna Jimenez[6], Guillermo Comach[7], Ivette De Rivera[8], Roberto Agudo[9], Ana E. Arango[10], Alma Barboza[11], Nicolas Aguayo[11], Tadeusz Kochel[1]

*[1]Naval Medical Research Center Detachment, Lima, Peru, [2]Direccion General de Epidemiologia, Lima, Peru, [3]Hospital Vozandes, Quito, Ecuador, [4]Hospital Materno Infantil San Francisco Solano, Buenos Aires, Argentina, [5]Hospital Infantil Manuel de Jesus Rivera, Managua, Nicaragua, [6]Hospital Nacional de Metapan, Santa Ana, El Salvador, [7]Laboratorio Regional de Diagnóstico e Investigación del Dengue y otras Enfermedades Virales, Maracay, Venezuela, [8]Universidad Nacional Autonoma de Honduras, Tegucigalpa, Honduras, [9]Servicio Departamental de Salud, Cochabamba, Bolivia, [10]Grupo de Inmunovirología, Universidad de Antioquia, Medellin, Colombia, [11]Asociacion Rayos de Sol, Asuncion, Paraguay*

Influenza is an acute, febrile disease that causes substantial morbidity. Type A influenza viruses are the major cause of influenza in humans and produce approximately half a million fatalities every year. Recent studies reveal an alarming increase in Neuraminidase Inhibitors (NAIs) resistant influenza A/H1N1 strains worldwide. We had previously reported that in Central and South America, until 2007, resistance to oseltamivir was not detected among influenza A/H1N1 samples. In this study, we have evaluated influenza viral resistance to NAIs in symptomatic patients throughout Central and South America during the three year period from 2006 to2008. Interestingly, our findings show, that the strains found in Europe between 2006 and 2008, are present now in Latin America suggesting a viral migration pattern from Europe to this region of the world, and even more importantly, that in this region, oseltamivir-resistant A/H1N1 viruses begun to be detected in 2008 and have become the vast majority (99%) of the A/H1N1 circulating viruses replacing previous circulating strains.

## 269

### EVALUATION OF THE USE OF EGYPTIAN *LEPTOSPIRA* ISOLATES IN THE MICROSCOPIC AGGLUTINATION TESTING (MAT) DIAGNOSIS OF LEPTOSPIROSIS

Clinton K. Murray[1], Michael R. Gray[2], Katrin Mende[3], Guillermo Pimentel[4], Tina Parker[4], Ahmed Samir[4], Bassem Abdel Rahman[4], **Duane R. Hospenthal**[1]

*[1]Brooke Army Medical Center, Fort Sam Houston, TX, United States, [2]United States Army Veterinary Command, Fort Sam Houston, TX, United States, [3]Infectious Disease Clinical Research Program, Bethesda, MD, United States, [4]NAMRU3, Cairo, Egypt*

The range of clinical manifestations of leptospirosis results in clinical presentations that overlap with other acute febrile illnesses (AFI) in tropical regions. This places an increased emphasis on the need for adequate diagnosis of this zoonosis. The gold standard for diagnosis is considered to be microscopic agglutination testing (MAT). Use of live antigens recovered from local patient cultures to develop an improved local MAT panel has been promoted. This study assesses the use of clinical cultures obtained from Egypt to develop a pathogen-specific MAT to screen serum obtained from patients who presented to a regional clinic with AFI. We utilized 11 isolates recovered from Egyptian human blood (Bataviae, Pyrogenes, Grippotyphosa, Pomona, Wolfii, Canicola) and animal (Patoc (cat), Grippotyphosa (rat), Pyrogenes (rat), Canicola (rat), Bataviae (dog)) samples to develop country-specific MAT. These live antigens were used to test serum obtained from 41 patients in the same region who presented with AFI and were confirmed infected by recovery of *Leptospira* in their blood. Criteria for positive MAT was a titer of $\geq$ 1:800. Testing of the 41 patient sera found 7 positive by PanBio ELISA using a cutoff of >11 units, but only one met criteria for leptospirosis by MAT. This sample had a PanBio ELISA >20 units and MAT positive at >1:12,800 against serovar Grippotyphosa. Culture for this patient grew *L. interrogans* serovar Pomona. Another patient grew *L. kischneri* serovar Grippotyphosa from culture, but had MAT titers of 1:400 against serovar Pyrogenes and Wolfii. Region-specific MAT was less reliable than PanBio ELISA for the diagnosis of leptospirosis among patients presenting with an AFI. Additionally, neither of the 2 patients described has MAT results that correlated with their recovered *Leptospira* serovar. Continued efforts are needed to develop testing methods to adequate diagnose acute infection that are also reflective of the infecting serovar.

## 270

### IGM ANTIBODIES AGAINST Q FEVER IN ACUTE FEBRILE PATIENTS IN ACCRA, GHANA

**Naiki Puplampu**[1], Shirley Odoom[1], Janice Tagoe[1], Engy Emil Habashy[2], Michael Wilson[1], Gregory Raczniak[3], Karl Kronmann[3], Edward Nyarko[4], Prince Agbenohevi[4], Moustapha Abdel Fadeel[2], Guillermo Pimentel[2], Kwadwo Koram[1]

*[1]Noguchi Memorial Institute for Medical Research, Legon, Ghana, [2]Naval Medical Research Unit-3, Cairo, Egypt, [3]Noguchi Memorial Institute for Medical Research/Naval Medical Research Unit-3, Ghana Detachment, Legon, Ghana, [4]37th Military Hospital, Accra, Ghana*

Malaria is endemic in Ghana and is mainly diagnosed based on clinical symptoms. The Ministry of Health estimates that over the past ten years, there have been 2-3 million cases of malaria each year. While typhoid fever is frequently recognized as another cause of febrile illness, it is less clear what burden other infectious diseases, such as Q fever have in Ghana. One hundred and ninety eight patients have so far been enrolled in an ongoing hospital-based acute febrile illnesses surveillance study in Accra. People presenting at the hospital with fever lasting two days or more and a temperature of >38°C are recruited. Patients with obvious focal clinical diagnosis and children under the age of 4 years are excluded. Every individual who meets the case definition provides written and informed consent before enrolment. This study was approved by the Noguchi Memorial Institute for Medical Research and Naval Medical Research Unit-3 IRBs. After filling a case record form for data on sex, age and recent exposure to certain animals, patients provide 7-10 ml of venous blood for serology, culture and malaria thick and thin film. The Panbio Diagnostics ELISA kit for *Coxiella burnetti* (Q fever) IgM was used to screen the sera and IgM antibodies to *Coxiella burnetti* were detected in 14 (7.1%) samples. Of these 14 positives, six were classified as confirmed, and eight as probable according to manufacturer's instructions. Not surprisingly, the clinical diagnosis was malaria in seven of the 14, of which 4 were smear positive cases. Other clinical diagnoses in these cases included bronchopneumonia (1), enteric fever (1), upper gastrointestinal bleed (1) and no diagnosis (4). The average duration of illness at presentation was 5.3 days (range 3 days to 12 days), and no patients died. These results indicate that *Coxiella burnetti* infections may be contributory to acute febrile illnesses presenting at Accra hospitals.

## 271

### EIGHT YEAR-STUDY OF HUMAN FASCIOLIASIS IN THE ANDEAN PERUVIAN REGION: A PUBLIC HEALTH PROBLEM

**Luis A. Marcos**[1], Angelica Terashima[2], Eduardo Gotuzzo[2]

*[1]University of Texas, Houston, TX, United States, [2]Instituto de Medicina Tropical Alexander von Humboldt, Lima, Peru*

Significant transitional research has been promoted in fascioliasis in Peru since 2001. The objective of this study was to systematically review published original articles that focus on basic and clinical research in fascioliasis and its impact in public health in Peru. The databases of MEDLINE, EMBASE, LIPECS and ScieLO Peru were searched from 2001 to 2008 to identify relevant articles published in English or Spanish. Results: A total of eighteen selected articles were published in Peru. Seventeen

were performed by our group from 2001-2008 (Institute of Tropical Medicine Alexander von Humboldt - IMTAvH). A total of 1877 cases (patients ranging from 2 to 75 years of age) were reported during this time period with both, acute and chronic infections. Almost 2/3 of the country has reported cases of human fascioliasis. The acute infection was described as a pentad syndrome characterized by fever, hepatomegaly, marked eosinophilia, right upper quadrant pain and hypodense track-like lesions by CT scan of liver. Patients with chronic infection were for the most part asymptomatic and counted for 90% of the cases. A few have life-threatening complications such as cholangitis, hepatic abscesses and biliary obstruction. Fas2-ELISA is a new, highly sensitive and specific serological test for acute infections. The Rapid Sedimentation Technique is by large the gold standard to diagnose the chronic infection. Triclabendazole has a cure rate of more than 90% for both phases of the infection. In basic research, the chronic infection leads to the development of liver fibrosis in animal models and depends on the intensity of infection. In 2008, the Ministry of Health, for the first time in Peru, implemented a national program to prevent and control *Fasciola hepatica* infection in endemic areas under the supervision of IMTAvH at Lima, Peru. In conclusion, human fascioliasis has become an important parasitic infectious disease of national importance in Peru. Based on our studies, future research is warranted along with control and prevention programs towards the eradication of this infection in the Andean Region and other endemic countries.

## 272

### DEVELOPMENT OF A QUANTITATIVE REAL TIME PCR TO ANALYZE THE EXPRESSION PROFILE OF TH1/TH2 CYTOKINES GENES IN A RABBIT MODEL OF FASCIOLIASIS

**Ana M. Espino**, Francheska Rivera

*University of Puerto Rico, School of Medicine, San Juan, Puerto Rico*

The analysis of cytokines profiles helps to clarify functional properties of immune cells, both for research and for clinical diagnosis. The quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) is becoming widely used to quantify mRNA expression from cells, body fluids, tissues, or tissue biopsies. Being a powerful and sensitive method it is usually used to quantify mRNA expression levels of cytokines, which normally are expressed at very low levels. Due to the crescent availability of a number of complete leukocyte expressed sequences tags (ESTs) as well as of complete genomic sequences most of quantitative RT-PCR (qRT-PCR) has been developed to quantify cytokines in mice and humans. In contrast, very few studies have been carried out to develop arrays that permit to quantify cytokines in domestic animals such as rabbit. Rabbit is a good model for studying a large variety of studies including vaccination and challenge with infectious organisms and in our laboratory it has been traditionally used as model for testing the efficacy of novel vaccine candidate against *Fasciola hepatica*. Although a number of leukocyte expressed sequences tags (ESTs) are available in the gene bank, no results of a qRT-PCR array specific for this specie have been published. In the current study we described a qRT-PCR array for six rabbit Th1/Th2 cytokines that was validated during the course of a vaccination trial with the novel *F. hepatica* antigen termed FhSAP2 that was challenged with *F. hepatica* infectious dose. This array led us to determine that protection conferred by the protein FhSAP2 in the rabbit model of fascioliasis is associated to a mechanism driven by the CD4+Th1 cells.

## 273

### *IN VIVO* IMAGING ON BILE-CHEMOTACTIC MIGRATION OF JUVENILE *CLONORCHIS SINENSIS* IN RABBITS

**Sung-Jong Hong**, Tae Im Kim, Won Gi Yoo, Hye Lim Kang, Ji Sung Jung, Byung Kook Kwak, Ju–Won Seok

*Chung-Ang University, Seoul, Republic of Korea*

The metacercariae of *Clonorchis sinensis* excyst in the duodenum and migrate with chemotaxis to bile in the bile duct. We were curious about how fast the *C. sinensis* newly excysted juveniles (CsNEJs) migrate up through the ampulla of Vater. The CsNEJs were labeled with $^{18}$F-fluorodeoxyglucose ($^{18}$FDG) by incubating in a maintaining media containing 2 mCi $^{18}$FDG at 37°C. After washing, radioactivity was measured using a gamma counter. Labeling efficiency was highest at 15 min incubation. Sensitivity of rabbits to cholecystokinin-8 (CCK-8), gallbladder contracting agent, was determined using $^{99m}$Tc-mebrofenin cholescintigraphy. After injecting 2 mCi $^{99m}$Tc-mebrofenin in 0.5 ml volume to the rabbits starved rabbits for 16 hrs, bile secretion was derived by intravenously injecting CCK-8 at dosage 20 ng/kg every 1 min, then dynamic image was taken every 1 min for 1 hr. The gallbladder was contracted 50% volume within 12 min of CCK-8 injection. A catheter was positioned in middle duodenum of the rabbit under anesthesia and 20 ng/kg CCK-8 was injected every 1 min over this experiment. *In vivo* images were collected using positron emission tomography- computed tomography (PET-CT). One CT image was scanned at starting point and dynamic PET scan was performed for 90 min with a 3-min acquisition per frame. Twelve min after CCK-8 injection, about 3,000 $^{18}$FDG-labeled CsNEJs were placed in duodenum through catheter. The CsNEJs were detected in the common bile duct 9 - 10 min after the CsNEJ injection, and kept migrating up peripheral capillary bile duct during early 30 min. A large number of adult *C. sinensis* were recovered from the rabbit livers 4 weeks after the CsNEJ infection. Collectively, the CsNEJs sensed bile in the duodenum and migrated up quickly with bile-chemotaxis into the bile duct.

## 274

### PROTEIN KINASES OF THE PARASITE *SCHISTOSOMA MANSONI*

**Luiza F. Andrade**[1], Lívia Avelar[1], Jerônimo Ruiz[1], Adhemar Zerlotini[2], Laila Nahum[1], Guilherme Oliveira[1]

*[1]Fiocruz - CpQRR, Belo Horizonte, Brazil, [2]UFMG, Belo Horizonte, Brazil*

In spite numerous efforts made to control schistosomiasis, transmission has not stopped. Schistosomiasis remains an important parasitic disease and represents a serious public health concern and a major economic problem in many developing countries. Current estimates are that 200 million individuals are infected and that the disease causes 280,000 deaths annually in sub-Saharan Africa alone. Praziquantel is the drug of choice for the treatment of schistosomiasis and the only one that is available for mass chemotherapy. However, there are problems with its use, including the decreased susceptibility of juvenile stages and the reports of drug resistance in different endemic regions. The identification of new drug targets is highly desirable. Eukaryotic protein kinases (ePKs) constitute one of the largest mammalian gene families and are key regulators of a wide variety of conserved cellular processes including cell cycle, cell growth and death, metabolism transcription, morphology and mobility, and differentiation. ePKs are currently vigorously investigated as potential drug targets in several disease models. The recent sequencing of the *Schistosoma mansoni* genome and large-scale transcriptome projects have provided the necessary information for the identification of protein kinases. We focus here on the content and diversity of protein Kinases present in *S. mansoni*. Computational searches, with the use of HMM, of the predicted *S. mansoni* proteome for ePKs reveled a total of 249 PKs. A highly curated manual annotation of *S. mansoni* genome supercontigs presenting positive hits was produced using Artemis and in house developed Perl scripts. A Phylogenetic tree was constructed to position the sequences relative to the nine estabilished ePK groups. Several sequences did not cluster whitin any group and 22 alternative splicing events were identified. Same groups have more members than what was observed for model organisms, such as STE and CMGC family protein kinases that are involved in regulating cell cycle control, differentiation and response to stress.

## 275

### *SCHISTOSOMA MANSONI* VACCINE CANDIDATE SCREENING BY BI-DIMENSIONAL WESTERN BLOT

**Fernanda Ludolf**, Rosiane da Silva-Pereira, Paola Patrocínio, Rodrigo Corrêa-Oliveira, Guilherme Oliveira

*Centro de Pesquisa Rene Rachou, Belo Horizonte, Brazil*

With the bidimension eletrophoresis technology it is possible to obtain near complete protein separation of many different kinds of cellular extracts. Using the 2D electrophoresis approach coupled to western blot, we aim at identifying novel antigens using different developmental stages of *Schistosoma mansoni* parasite that are in contact with the immunological system of the vertebrate host: schistosomula, cercarie, adult worms and eggs. To probe the 2D westerns, sera of infected individuals and of individuals resistant to infection (normal endemics, NE), were used and the pattern of spots recognized compared. Antigens recognized by both sera and particularly those recognized exclusively by NE sera of were be selected and identified by mass spectrometry. Results obtained thus far indicate that the pattern of adult worm protein antigens recognized by NE sera has clear qualitative and quantitative differences from the pattern produced by the sera of infected individuals. Many of the spots observed were recognized by both antisera. The profile of schistosomula and adult worm proteins indicate clear differences in their repertory of antigenic proteins. We also observed that there was no correlation between the amounts of proteins present in the sample and their antigenicity. Many of the proteins identified until now are proteins already related as immunogenic, yet some other proteins still not even characterized. These proteins are already in process to be expressed for further antigenic characterization and protection experiments in the mouse model. The proteins identified by this project could represent new targets for the development of a vaccine for the schistosomiasis.

## 276

### RURAL TOURISM AND SCHISTOSOMIASIS: THE GEOGRAPHIC INFORMATION SYSTEM AS A TOOL FOR IDENTIFICATION OF PRIORITY AREAS FOR INTERVENTION

**Ronaldo G. C. Scholte**, Omar S. Carvalho, Guilherme C. Oliveira, Martin J. Enk

*Centro de Pesquisas René Rachou/Fiocruz, Belo Horizonte, Brazil*

Schistosomiasis mansoni in Brazil was considered a mainly rural endemic disease, affecting the low-income population with a tendency to spread into the periphery of big urban centers during the last decades. As recently described rural tourism of middle- and upper-class urban residents leads to exposure of this section of the population to infection with this disease during leisure activities in endemic rural areas. The boom of this type of tourism is identified as a contributing factor to the dissemination of the infection with *Schistosoma mansoni*, which is little perceived. This situation gave reason to use Geographic Information System (GIS) and Remote Sensing (RS) to study rural tourism areas as it is the case with The "Estrada Real" project. The "Estrada Real" with an extension of about 1,400 km is one of the largest and most ambitious Brazilian tourism projects, involving 178 municipalities in the states of Minas Gerais (163), Rio de Janeiro (08) and Sao Paulo (07). In Minas Gerais, the Estrada Real passes through 29 municipalities with known prevalence levels of schistosomiasis mansoni ranging from 0.06 to 28.2%, most of them with a strong appeal to rural tourism. This is a worrying situation because tourists are attracted to this region from all over Brazil, including areas without schistosomiasis transmission, and from other countries, free of the disease. Using the GIS/RS techniques in combination with spatial statistics a map was developed, which allows prevalence estimates for each of the 163 municipalities, indicating 23 (13%) of them with a ratio of positivity greater than 10% and 140 (87%) with less than 10%. This approach facilitates the identification of priority areas for intervention, making control and surveillance efforts more efficient. In this context this methodology also may serve to direct preventive measures such as environmental education / health awareness programs especially in regions with tourism as it is the case with the "Estrada Real".

## 277

### COMPARISON OF VECTOR SNAILS OF SCHISTOSOMIASIS IN TWO SITES ALONG THE WEIJAH LAKE IN GHANA

**Naa Adjeley Tawiah**[1], Godfred Futabi[2], Kwadwo K. Frempong[3], Joseph Otchere[3], Dominic Edoh[2]

*[1]University of Ghana Medical School, Microbiology Department, Accra, Ghana, [2]Zoology Department, University of Ghana, Accra, Ghana, [3]Noguchi Memorial Institute for Medical Research, Accra, Ghana*

Schistosomiasis is one of the neglected tropical diseases in Ghana which is transmitted through contact with snails infected with cercariae in water bodies. Due to this, the disease is endemic in communities along banks of infected water bodies in Ghana. This study compared the distribution of vector snails at two endemic sites of the Weijah Lake which is an infected water body. Cercariae infection of snails from sites of high human activities and low human activities where compared to find out the effect of human activities (environmental changes) on snail infection. As part of the study, different snail species were collected from Galilee and Mahem which are endemic communities along the banks of the Weijah Lake. All snail samples were collected and sorted out using Kpikpi, Kristensen and Mandahl-barth keys. Snails which were infected with cercariae where detected after continuous exposure of live snails to bright light for about 25 minutes till emergence of cercariae. In all, four species of snails were collected including; Melanoides sp, Bulinus sp, Biomphalaria and Physa sp. Mahem, a community with dense vegetation and less human activity recorded 0.44% and 0.42% of Bulinus sp and Biomphalaria sp respectively. On the other hand Galilea described as having less dense vegetation and increased human activity recorded 0.373% and 0.348% respectively. Both sites had cercariae infection for Bulinus and Biomphalaria sp which in Ghana are the two main vector species of Schistosomiasis. Identifiable keys such as shell shape, sculpture, arrangement of whorls, aperture opening, and other keys described aided snail identification. Human activity and vegetation distribution have impact on vector snail distribution. Cercariae infection plays an important role in Schistosomiasis infection.

## 278

### MULTICOMPONENT REACTION CHEMISTRY FOR NEW SCHISTOSOMIASIS DRUGS

**Kareem Khoury**

*University Of Pittsburgh, Pittsburgh, PA, United States*

Schistosomiasis is a parasitic trematode infection endemic to tropical areas affecting more than 200 million people worldwide. Symptoms of disease include abdominal pain, cough, diarrhea, fever, fatigue, pulmonary hypertension, an enlarged liver and spleen, lowered immunity to other diseases, cancer and developmental deficits in children. Currently praziquantel (PZQ) is the only drug used to treat schistosomiasis on a large scale. Despite its use for over two decades, the molecular mechanism of action is still unknown. As the severity of this disease warrants a non-traditional approach, we are using a combination of multicomponent reaction (MCR) chemistry and cell biology to gain a better understanding of both schistosomal biology and the mechanism of praziquantel on the tremetode. Pursuant to this aim, our lab has first set forth to derive a faster and cheaper access to PZQ and its derivatives using MCR chemistry. A successful conversion of four commercially available starting materials to PZQ in two steps using the known four-component Ugi reaction followed by the Pictet-Spengler cyclization is shown along with synthesis of more then 100 PZQ derivatives using the same methods in high yields. Additionally, a library of dyes was created using MCR chemistry, which was then used to examine the parasite on both the cellular and whole organism level. The starting materials of the MCRs were altered so that the final products gave a wide variety of size, polarity, chirality, lipophilicity

and UV absorbance. We also incorporated PZQ, into the starting material of our dye library so as to gain a better understanding of the specifics of drug action on the schistosomes. After injection, the schistosomes were visualized on a cellular and sub-cellular level using confocal microscopy. This process can allow us not only to understand the mechanism of PZQ better but also to develop new drugs to be used in the case of PZQ resistance. This new technique could also allow us look at mechanism for other unmet medical needs in neglected tropical diseases.

## 279

### IMMUNOLOGICAL MEMORY IN OFFSPRING BORN TO BABOONS (*PAPIO ANUBIS*) INFECTED WITH SCHISTOSOMIASIS *MANSONI*

**David Langoi**[1], Thomas Kariuki[1], Michael Gicheru[2], Idle O. Farah[1], Hans_Erik Carlsson[3]

*[1]Institute of Primate Research, Nairobi, Kenya, [2]Kenyatta University, Nairobi, Kenya, [3]Uppsala University, Uppsala, Sweden*

This study was undertaken to test the hypothesis that offspring born to *Schistosomiasis* infected mothers resist a challenge infection more and experience less severe pathology than offspring born to non infected mothers we studied the immunopathogenic impact on baboon fetus arising due to a prenatal exposure to *Schistosoma mansoni.* We determined whether the immune system of fetus will be sensitized or tolerized to a subsequent infection later in life. Five (5) female adult baboons of similar weights were infected with *600 Schistosoma mansoni* cercariae and monitored to develop a chronic infection. A second group (N=5) of uninfected female baboons served as parallel controls. All baboons' estrus cycles were synchronized with hormonal therapy and animals bred to obtain age matched offspring. Five (5) offspring were delivered by cesarean section from the infected baboons and five (5) offspring from the naïve baboons. All infants were challenged with *600* cercariae at 1 year of age. Blood was obtained at challenge, 3, 6 and 10 weeks post challenge infections. The specific peripheral blood mononuclear cell proliferative responses (PBMC) to *Schistosoma egg (SEA)* and *worm (SWAP)* was measured by stimulation of cells in invitro cultures. Interferon γ (IFN) in the cell culture supernatants was assayed by sandwich ELISA and parasite specific IgG/M antibodies were measured by indirect ELISA. The baboons were perfused by portal perfusion at 10 weeks post challenge for recovery of adult worms and assessment of pathology of tissue. The PBMC, IFN and IgG responses to *SEA and SWAP* were significantly higher ($p<.05$) at 10 weeks after challenge in infants born to infected baboons. The adult worm counts did not differ in infants. The liver granulomas were significantly smaller ($p<.05$) in the infants born to infected baboons. In conclusion, we showed that prenatal exposure to *Schistosoma mansoni* leads to immune sensitization in the fetus and also less severe liver pathology in subsequent infection. The findings have implications for future prevention measures to disease.

## 280

### *SCHISTOSOMA MANSONI*: HOW TO MAKE A FEMALE

**Christoph Grunau**, Jérôme Boissier, Céline Cosseau, Sophie Beltran, David Duval, Julien Portela, Benjamin Gourbal, André Theron, Guillaume Mitta

*University of Perpignan, Perpignan, France*

The human blood fluke *Schistosoma mansoni* is the causative agent of one of the most severe human schistosomiasis. About 200 million people throughout the subtropical and tropical world are infected with one of the Schistosoma species. The parasite originates probably from the east-african lake area, but with was imported with slave trade into the American continent. It is today endemic not only in sub-Saharan Africa but also on the east coast of South America and on the Caribbean Islands. In contrast to most digenetic trematodes, schistosomes are dioecious with pronounced sexual dimorphism. Sex chromosomes have been identified (male ZZ, female WZ) but the identification of sex-specific genomic regions remains elusive. We have used next-generation sequencing to identify unique and repetitive sequences that are female-specific. Our data support the view that sex determination of *S. mansoni* is based on repeat-induced heterochromatization of the W chromosomes, i.e. it relies on epigenetic and genetic components. These findings will be presented with relation to the evolutionary history of schistosomes, and epigenetics as an emerging drug target.

## 281

### CLONING, CHARACTERIZATION, AND EXPRESSION OF *SCHISTOSOMA MANSONI* MICRORNAS

**Ms. Mariana C. Simoes**

*Oswaldo Cruz Foundation (Fiocruz) - Centro de pesquisas Rene Rachou (CPqRR), Belo Horizonte, Brazil*

Small RNAs play a crucial role in regulating development and controlling expression of many animals' genes by targeting mRNAs and triggering either translation repression or RNA degradation. microRNAs (miRNAs) are widespread in eucaryotes and several hundred of miRNAs have been identified. In schistosomas, the full set of miRNAs and their expression patterns during development are still poorly understood when compared to the other species. In this study, we describe the cloning and expression of novel miRNAs from Schistosoma mansoni. By high-throughput sequencing of small-RNA cDNA libraries from adult worms, we cloned 211 sequences candidates to be miRNAs in the *S. mansoni* genome. Northern blot was used to measure the expression of selected miRNAs during schistosomula and adult worm stage. Analysis of stage distribution of these miRNAs showed that some are present ubiquitously, while others are stage-specific. Expression of 11 novel miRNAs out of the 30 most abundance from the library and 3 out of the 5 miRNAs previously published in *S. japonicum* have been confirmed by Northern blotting, which indicated the cooperative nature of the miRNA expression with their possible function in the regulation of various cellular processes.

## 282

### STOCHASTIC MODELING OF SCHISTOSOMIASIS *JAPONICUM* TRANSMISSION

**Yao Ning**, Kin On Kwok, Steven Riley

*School of Public Health, The University of Hong Kong, Hong Kong, China*

The incidence of schistosomiasis *japonicum* infection in China and the Philippines often varies substantially from village-to-village, even within a small geographical area. Much of this variability is likely driven by fundamental differences in the transmission potential at these sites. However, with such small populations involved, we hypothesize that some of the variability may be driven by pure chance (demographic stochasticity). Therefore, we present a stochastic transmission model of Schistosomiasis *japonicum* at the village-level with the objective of exploring the feasible role of intrinsic variability of transmission on disease incidence over time. We developed a stochastic model to simulate schistosomiasis transmission in a closed environment with constant snail population. The model tracked individual worm burden and infected snails at the village-level. The model was structured to allow rates of transmission from snails to human to vary between different risk groups. The model also incorporated density-dependent effects of worm establishment and fecundity. We simulated transmission of Schistosomiasis followed by mass chemotherapy. We simulated epidemics of schistosomiasis infection running to an endemic state. For an illustrative set of parameter values, there was some stochastic variation around the endemic state. However, immediately after mass chemotherapy, there was substantial stochastic variation in the prevalence of infection. As the system returned to its endemic state, these degree of stochastic variation was reduced. Field studies of interventions designed to reduce schistosomiasis should be run in multiple communities and with sufficiently long follow-up times to ensure that estimates of community-level efficacy are not unduly influenced by stochastic chance.

## 283

### THE *SCHISTOSOMA MANSONI* RACK1 IS EXPRESSED IN THE GONADS AND IS LIKELY INVOLVED IN EMBRYOGENESIS

**Livia Avelar**[1], Colette Dissous[2], Christoph Grevelding[3], Guilherme Oliveira[1]

*[1]Rene Rachou Institute of Research, Oswaldo Cruz Foundation, Belo Horizonte, Brazil, [2]Institut Pasteur, Lille, France, [3]Heinrich-Heine-Universität, Düsseldorf, Germany*

Schistosomiasis affects more than 200 million individuals in 75 countries; 600 million live at risk. Its impact on health, monitored by the WHO, is very severe and the main control strategy is mass treatment with the sole drug available, praziquantel. Reports of resistance make the development of new drugs highly desirable. Key molecules involved in signal transduction are extensively studied as potential drug targets for a variety of pathologies. In order to discover putative new drug targets we propose to identify and characterize pathways that play key roles in signal transduction pathways important for the development of schistosomes. We have characterized the first RACK1 gene in S. mansoni, SmRACK1. Our findings suggest that SmRACK1 may play a critical role in signal transduction pathways and embryogenesis of S. mansoni. Structural analysis indicated that SmRACK1 exhibits features which are WD-40 Repeat family, containing seven tandem WD-40 motifs. SmRACK1 genomic sequence was assembled from sequences generated by Sanger Institute. A total of three exons and two introns were identified. Screening the *S. mansoni* genome sequences with the SmRACK1 cDNA sequence showed that only one copy of the gene is present in the genome. The complete cDNA of SmRACK1 contains an ORF of 948 bp, encoding a protein with 315 amino acids and a theoretical molecular weight of 34,6 kDa. SmRACK1 orthologues were identified in 8 different Schistosoma species. The differential expression of SmRACK1 mRNA during the *S. mansoni* life cycle was investigated using quantitative RT-PCR. SmRACK1 is expressed at all life cycle stages at different levels and SmRACK1 mRNA was at the highest levels in miracidia. SmRACK1 interact with SmPKC1 *in vitro*. GST-SmRACK1 bound to glutathione-sepharose beads was incubated with equal quantities of SmPKC1 generated from an *in vitro* transcription-translation system. A major band of 136,6 kDa was detected by Western blot carried out with an antiserum directed against GST-tail of pGEX-4T-3 vector. Localization studies by in situ hybridization revealed that SmRACK1 is predominantly expressed in the reproductive organs such as the ovary and vitellarium of the female. These findings suggest that SmRACK1 may play a critical role in signal transduction pathways and embryogenesis of S. mansoni.

## 284

### *SCHISTOSOMA MANSONI* PKA: A POTENTIAL NEW DRUG TARGET

**Brett E. Swierczewski**, Stephen J. Davies

*Uniformed Services University of the Health Sciences, Bethesda, MD, United States*

Protein kinases represent novel drug targets for the treatment of diseases caused by eukaryotic pathogens such as helminth parasites. We therefore explored the anti-parasite potential of targeting cAMP-dependent protein kinase (PKA) enzymes in *Schistosoma mansoni*. Examination of the *S. mansoni* genomic sequence database identified sequences of four putative PKA genes. Using reverse transcriptase-PCR and RACE, transcripts from two distinct PKA genes were identified in adult *S. mansoni* cDNA, one of which is expressed as two distinct splice variants that utilize different exons at the 5′ end (SmPKA-C). Western blot analysis of adult *S. mansoni* proteins, using a polyclonal antibody directed against conserved sequences of the PKA $\alpha$ catalytic subunit, identified several protein species with expected molecular weights of PKAs. PKA activity was detectable in adult *S. mansoni* lysates at various nanogram concentrations, confirming that *S. mansoni* worms express active PKAs. Further, schistosome PKA activity was significantly inhibited and activated by commercially available PKA inhibitors and agonists. Three PKA inhibitors were shown to have schistosomocidal effects on adult worms *in vitro* at various micromolar concentrations within four hours to six days. RNA interference experiments using SmPKA-C dsRNA significantly decreased transcription and produced lethality in 50% of treated adult worms while decreasing overall SmPKA activity by 50% in surviving worms. Further studies revealed that *S. haematobium* and *S. japonicum* cDNA express nearly identical PKA C homologues to those of *S. mansoni*. These data suggest that inhibitors of PKA have potential as novel chemotherapeutics for the treatment of schistosomiasis and other helminth infections.

## 285

### DETECTION AND DISCOVERY OF VIRAL PATHOGENS ASSOCIATED WITH ACUTE PEDIATRIC RESPIRATORY ILLNESS IN NICARAGUA

**Nathan Yozwiak**[1], Aubree Gordon[1], Saira Saborio[2], Peter Skewes-Cox[3], Guillermina Kuan[4], Angel Balmaseda[2], Eva Harris[1], Joseph L. DeRisi[3]

*[1]University of California, Berkeley, CA, United States, [2]Departamento de Virología, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud, Managua, Nicaragua, [3]University of California, San Francisco, CA, United States, [4]Centro de Salud Sócrates Flores Vivas, Ministerio de Saludornia, Managua, Nicaragua*

Monitoring the international emergence of human pathogens is critical to global public health efforts aimed at preventing and treating infectious diseases. Viral surveillance studies yield important information about the prevalence and seasonality of circulating viruses and the possible existence of novel species, yet few large epidemiologic studies monitoring for respiratory viruses have been conducted in tropical settings, as they have in temperate countries. Since respiratory diseases are a major cause of morbidity and mortality worldwide, correctly identifying the agents associated with respiratory infections is critical for guiding policy, treatment, and prevention strategies. Recently developed unbiased methodologies, such as pan-viral microarrays and high-throughput sequencing, have improved viral detection capabilities, making large-scale broad-spectrum viral surveys and novel viral discovery more feasible than ever before. This study sought to use a viral microarray (Virochip) to delineate the spectrum of viral pathogens in a developing country tropical environment with respect to acute pediatric respiratory illness and to utilize the data provided by the extensive screening as a platform for the discovery of novel or divergent human viral pathogens. We then employed ultra high throughput sequencing to address samples refractory to RT-PCR and Virochip analysis. We utilized a prospective cohort study of influenza-like illness (ILI) involving ~3,800 children aged 2-12 years old in Managua, Nicaragua, from June 2007 to June 2008. We used the Virochip to analyze nasal and throat swab specimens determined to be negative by RT-PCR for several common respiratory viruses. We detected a virus in 44 of 139 samples (32%), of which 30 of 44 (68%) were Picornaviruses and 5 of 44 (11%) were human Metapneumovirus. Specific Picornavirus RT-PCR subtyping of the VP4/VP2 region revealed extensive diversity, including 57% of Rhinoviruses belonging to the newly discovered Group C. We also detected a highly divergent Enterovirus in 5 of 212 (2.4%) samples screened by RT-PCR. Sequence from the VP1 region is only 71% identical to the nearest relative, yet 96% identical within the five isolates. We used ultra-deep Solexa sequencing to recover additional divergent genomic regions. Our findings demonstrate a high rate of Picornavirus detection in acute pediatric ILI cases in Nicaragua with extensive genetic diversity.

## 286

### MOLECULAR EVIDENCE OF HANTAVIRUS DOBRAVA SPILLOVER INFECTION IN BANK VOLE

**Miša Korva**[1], Darja Duh[1], Nataša Knap[1], Tomi Trilar[2], Tatjana Avšič-Županc[1]

*[1]Medical Faculty, Ljubljana, Slovenia, [2]Slovenian Museum of Natural History, Ljubljana, Slovenia*

Hemorrhagic fever with renal syndrome and hantavirus cardiopulmonary syndrome are complex human diseases characterized by different set of symptoms. Despite the differences in the course of both disease syndromes, the causative agents are hantaviruses. Hantaviruses are worldwide distributed zoonotic agents maintained in nature by evolving tight relationship with their specific rodent carriers. It is well known that each hantavirus is associated with a specific rodent host or few closely related host species. In Slovenia, HFRS is caused either by Puumala or Dobrava hantavirus and the severity of the disease manly varies depending on a particular virus involved. Both hantavirus rodent carriers, *Apodemus flavicollis* (DOBV carrier) and *Myodes glareolus* (PUUV carrier), are widely spread throughout the country. As their natural biotopes are overlapping, it is not surprising that different hantaviruses are found in the same endemic region. In year 2007 our annual rodent trapping was conducted in July at Savinjska region (location Vransko), which is endemic for hantaviruses and tick-transmitted diseases. Using Sherman type live traps, 50 rodents were caught in three consecutive nights. Forty two were identified as *A. flavicollis* and 8 as *M. glareolus*. Using multiplex one-step real time RT-PCR assay for specific diagnosis of DOBV/SAAV and PUUV, 10 RNA samples were found positive for DOBV. Nine of them were isolated from *A. flavicollis* spleen and one from *M. glareolus* spleen. Partial S segment sequences were recovered from all amplicons. Nucleotide sequence analysis confirmed the presence of DOBV in all of them, with the 99,1% to 100% identity among the isolates. Molecular evidence of Dobrava virus outside its primary host reservoir or outside closely related species questions whether the spillover events are far more common and important as previously thought. In addition, in locations like Vransko, where biogeographic factors allow frequent spillover, the simpatry might play more important role than host relatedness. Because spillover infection enables natural reassortment and origination of new hantavirus species, this might be also important in regards to public health.

## 287

### PRECIPITATING AND NEUTRALIZING ANTIBODY RESPONSE TO HIGHLY PATHOGENIC AVIAN INFLUENZA, NIGERIA

**Clement A. Meseko**, Anthony Egbuji, Folorusho D. Fasina, Agnes T. Oladokun, Stella Idachaba

*FAO Regional Lab for Avian Influenza, National Veterinary Research Institute, Jos Plateau State, Nigeria*

Highly Pathogenic Avian Influenza is a very severe disease of birds caused by type A influenza virus and was reported for the first time in Nigerian in February 2006 where it caused almost 100% mortality in affected poultry flocks. In a deliberate effort to detect the disease early in order for control measures to be instituted, active and passive surveillance activities were carried out in 36 states of the Federation including the capital. Sera specimens were collected from chickens, turkeys, ducks, guinea fowls and pigeons from all the agro-ecological zones of the country in poultry farms, wetland areas and live bird markets. A total of 9,167 sera specimens collected between 2006 and 2008 were analyzed by Agar Gel Immuno diffusion (AGID) and Heamaglutination Inhibition (HI) tests for precipitating and neutralizing antibody to HPAI respectively according to OIE protocols. Six (0.082%) of the samples collected in live bird market in the Western part of the country in 2006 and 2007 were positive for precipitating antibody to Flu A and 1(0.054%) sample collected in a goose also in a LBM in the North eastern part of the country in 2008 was positive for neutralizing antibody to H5.The high morbidity and mortality rate of HPAI in poultry birds and other species results in deaths within 24 to 72 hours post infection which is less than the time required for seroconversion to most viral infections as shown in the low antibody response in this study which confirmed the highly pathogenic nature of the H5N1 virus that circulated in Nigeria during the period., the results also affirm the role of LMBS in the epidemiology of Avian influenza in Nigeria.

## 288

### PROMOTING BAMBOO SKIRTS FOR DATE PALM SAP COLLECTION IN BANGLADESH: A COMMUNITY INTERVENTION TO PREVENT NIPAH VIRUS TRANSMISSION

**Nazmun Nahar**, Utpal Kumar Mondal, Emily S. Gurley, M. Jahangir Hossain, A. K. Khan, Elizabeth Oliveras, Rouha Anamika Sarkar, S.M. Azad, Rebeca Sultana, M.S. Khan, Stephen P. Luby

*International Center for Diarrhoeal Disease Research, B, Dhaka, Bangladesh*

Consumption of raw date palm sap is common in rural Bangladesh. Drinking raw date palm sap contaminated with saliva or urine from infected fruit bats has been identified as an important mode of Nipah virus transmission to humans. A previous study showed that bamboo skirts are inexpensive and prevent bats from accessing the sap. The aim of this study was to assess the effectiveness of low compared to high intensity interventions to promote the use of bamboo skirts among date palm sap collectors (*gacchis*). From November 2008 to March 2009, we conducted a high intensity intervention in two villages and a low intensity intervention in two other villages and compared outcomes between the two groups. For the high intensity intervention we spent 160 hours including 7 meetings and 4 bamboo skirt making training sessions while in the low intensity intervention we spent half that amount of time by conducting 4 meetings and 2 training sessions to explain how to make and use the bamboo skirt and potential benefits of use. We documented people's comments on the bamboo skirt, followed up with all *gacchis* and tree owners in both intervention areas once a month for three months, observed the use of skirt when any *gacchi* reported it's use, and conducted 57 in-depth interviews to understand the reasons for using or not using bamboo skirts. In the high intensity intervention area 75% (48/64) and in low intensity intervention area 61% (53/87) of *gachis* made and used a skirt of bamboo or other materials like jute stake, weed plants, polythene sheets and gunny bags. There was no difference between the two intervention groups. (P=0.100). When *gacchis* who did not make a skirt wanted to drink raw sap, they borrowed skirt from other *gacchis*. Multiple family members were involved in skirt making. In conclusion, high proportion of all *gacchis* included in the intervention made and used *skirts* regardless of intervention intensity, suggesting that this is a feasible intervention. To effectively implement this intervention at large scale throughout the date palm sap collection region we need to know how we can maximize the cost effectiveness of bamboo skirt making and its promotion. We also have to learn more about potential advantages of using different materials for skirts making and if they are effective in keeping bats out of date palm sap.

## 289

### HUMAN MONKEYPOX GENOMIC DIVERGENCE AND DETERMINANTS OF PATHOGENICITY

**Stephen K. Gire**[1], Anjan Purkayastha[1], Jay Goff[2], Vicki Olsen[2], Inger Damon[3], John Huggins[2], Jean-Jacque Muyembe[4], Lisa Hensley[2], Kate Rubins[1]

*[1]Whitehead Institute for Biomedical Research, Cambridge, MA, United States, [2]United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, United States, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States, [4]Institut National du Reserche Biomedicale, Kinshasa, The Democratic Republic of the Congo*

Monkeypox (MPX) infection causes severe disease in both humans and non-human primates (NHP). Due to the discontinuation of Vaccinia vaccination upon global smallpox eradication, MPX has re-emerged

as a naturally occurring human pathogen. However, little is known about the relationship of MPX genomic and transcriptional variation to pathogenesis of the disease. To better understand the viral genetic factors that play a role in pathogenesis and the extent of genomic divergence, we analyzed MPX genomic and transcriptomic differences in both a NHP model and human disease in the Democratic Republic of Congo (DRC). 18 Cynomolgus monkeys were experimentally infected with MPX and examined over a 10-day period to investigate the effect of changes in viral genotypes on dissemination and pathogenesis of infection. We observed high virus titers in major organ systems and in circulating blood. We developed methods to sequence viral genomes directly from clinical material from NHPs and tested whole blood, peripheral blood mononuclear cells, serum, organ homogenates, scabs and throat swabs. We were able to obtain complete viral genome sequences and are currently mapping genetic variation as it relates to spatial and temporal distribution and pathogenesis during MPX replication. We sequenced approximately 8.9 million reads and mapped 890,250 reads uniquely aligning to the MPX genome, achieving an average coverage of 178-fold. We were also able to reconstruct the MPX genome, independent of a genomic scaffold with which to do the assembly. In addition we designed a novel tiling microarray in which both strands of the 200kb MPX genome were tiled providing 6x coverage per nucleotide. We developed a base-pair resolution map of the MPX transcriptome for several different strains with differing pathogenicities. The viral transcriptomes showed a distinct temporal regulation and species-specific expression of viral gene subsets related to pathogenic differences between clades. In order to compare NHP data with human MPX, we have established a study site in DRC, and collected clinical material from over 140 cases of human MPX. These results represent the first study of the pathogenesis of MPX utilizing next generation, massively parallel, deep sequencing and provide a platform for analysis of rare genotypes using clinical material as well as in-depth investigations of viral genomic divergence using animal models and human disease samples.

## 290

### RELATIVE ROLES OF TLRS AND RNA-HELICASES IN IMMUNE RECOGNITION OF RIFT VALLEY FEVER VIRUS

**Megan Ermler**, Heidi Page, Lewis Yuan, A. Desiree LaBeaud, Charles King, James Kazura, Amy Hise

*Case Western Reserve University, Cleveland, OH, United States*

Rift Valley fever virus (RVFV) is a zoonotic pathogen endemic to regions of Africa and the Arabian Peninsula. In the most severe cases, RVFV infection can cause retinitis, encephalitis, or hemorrhagic fever. The innate immune response to RVFV is suspected to be important in viral clearance but is still poorly defined. In animal models of RVFV infection, a strong protective role has been identified for type I interferon responses. In human infection, a delayed onset of interferon production is associated with the more severe forms of RVFV-induced clinical disease. Members of the Toll-like receptor (TLRs) and RNA helicase families constitute germ-line encoded pattern-recognition receptors (PRRs) capable of detecting viral PAMPs (pathogen-associated molecular patterns) and stimulating type I interferon responses. In this study, HEK cells expressing specific Toll-like receptors (TLRs) were transiently transfected with luciferase reporter plasmids of anti-viral genes of interest, such as nuclear factor kappa beta and interferon β, then stimulated with an attenuated strain of RVFV, MP-12. Anti-viral signaling pathways for RVFV were determined by using TLR-transduced HEK cell lines, dominant negative constructs to inhibit RNA helicases, and chemicals that interfere with endosomal signaling. These studies offer a starting point for characterizing host response during RVFV infection. Innate PRR utilization by RVFV was confirmed with studies using immune cells from TLR and MAVS (a common adaptor for helicase signaling) gene knockout mice.

## 291

### DETECTION AND QUANTIFICATION OF HUMAN HERPESVIRUS 8 IN SAMPLES OF PATIENTS WITH AIDS-ASSOCIATED KAPOSI'S SARCOMA BY REAL TIME PCR

**Paula Renata Machado**, Kleber Farias, Benedito Fonseca

*University of São Paulo, Ribeirão Preto, Brazil*

Kaposi's sarcoma (KS) is the most common neoplasm of AIDS patients. In 1994, a new herpesvírus, Human Herpesvirus 8 (HHV-8), was identified in HIV-related KS tissue, and has since been associated with KS-AIDS. The measurement of the viral load has been crucial to the diagnosis of active infection in the study of herpesviruses. The aim of this study was to design and establish a quantitative real-time PCR assay for routine diagnosis of HHV-8 infection in samples of human immunodeficiency virus (HIV) infected individuals with Kaposi's sarcoma assisted on the Hospital of the School of Medicine of Ribeirão Preto. In all cases, KS diagnosis was confirmed by routine histological examination. Samples collected from AIDS-KS patients consisted of blood samples and skin biopsies, all of them collected at the time of diagnosis. DNA was extracted using QIAamp DNA mini kit. The 211bp HHV-8 DNA fragment was cloned into the pDRIVE according to the manufacturer's instructions. This plasmid, containing part of HHV-8 ORF-26, was used on the assay standardization, and plasmid concentration was determined by spectroscopy. The Sybr Green (QIAGEN) reaction was performed in a final volume of 50µL using primers KSIN1/KSIN2. The assay shows a wide range of detection, ranging from 10(1) to 10(6) viral genome equivalents/reaction. Sample analysis show that mean HHV-8 viral load in skin biopsies was $10^4$ copies/mg of tissue and mean viral load on blood samples was $10^5$ copies/mL. The Sybr Green assay developed for the quantification of HHV-8 DNA is sensitive and the results indicate that the HHV-8 viral load is readily determined with real-time PCR and can provide clinically useful information on the follow-up of these patients, such as response to chemotherapy.

## 292

### SURVEILLANCE OF AVIAN INFLUENZA IN THE LIVE BIRD MARKETS OF PERU

**Ana Patricia Mendoza**[1], Bruno Ghersi[1], Marcela Uhart[2], Tadeusz Kochel[1], Joel Montgomery[1], Donald Brightsmith[1]

*[1]NMRCD, Washington, DC, United States, [2]Wildlife Conservation Society, New York, NY, United States*

Live-bird markets are common in almost every major city throughout Peru. In these settings, the mixture of highly stressed birds, domestic and wild, kept in unsanitary and over-crowed conditions may lead to enhanced transmission of a variety of diseases including avian influenza (AI). Avian disease surveillance is carried out periodically by the Minister of Agriculture in Peru; however, it is restricted primarily to large-scale domestic poultry operations. Currently, surveillance in backyard poultry and among wild and caged birds in local markets is insufficient. The goal of our surveillance activity was to monitor and identify pathogens circulating among caged birds in live-bird markets throughout Peru. We surveyed markets in three cities of Peru since March 2007. Caged bird populations at these markets were enumerated, species were recorded and husbandry practices were noted. Oral and cloacal swabs were collected from manually restrained birds. Fresh fecal sample swabs were obtained from birds of small body size and from birds of which we were unable to handle. Swab samples were collected annually from one bird per species per cage at each market stall. AI isolation was attempted in cell cultures at the Naval Medical Research Center Detachment, and by inoculation in embryonated eggs at the Universidad Nacional Mayor de San Marcos. Samples were collected from 469 domestic poultry, exotic and other caged birds - primarily parrots and passerines. All samples collected to date have been negative for evidence of AI and other avian pathogens; however, collections and testing are still ongoing. Sampling among additional coastal cities will initiate in June 2009. Although Peru is still free of highly pathogenic avian influenza, low pathogenic viruses have been isolated from migratory birds

along the Peruvian coast. Although we have not yet registered migratory species in our survey, there is historical evidence indicating that these species are periodically introduced into these markets for trade.

## 293

### HUMAN INNATE IMMUNE RESPONSE TO RIFT VALLEY FEVER VIRUS INFECTION

**Angelle Desiree LaBeaud**, Heidi E. Page, Megan E. Ermler, Amy G. Hise

*Case Western Reserve University, Cleveland, OH, United States*

Rift Valley fever virus (RVFV), a member of the family Bunyaviridae and Category A bioterrorism agent, is endemic to semi-arid regions of Africa and the Arabian Peninsula and is a significant threat to human and animal health. Animal models of RVFV infection have identified a strong protective role invoked by type I interferon (IFN) responses; likewise in human infection, delayed onset of IFN production is associated with more severe clinical manifestations of disease. The segmented negative sense RNA genome of RVFV codes for a non-structural protein, NSs, which has been shown to interfere with the ability of the host to mount an adequate IFN response. To gain a better understanding of human RVFV infection, we investigated the innate immune response to RVFV infection, the permissiveness of host cells to RVFV infection and the relationship between the two. Using an attenuated vaccine strain of the virus, MP-12, as well as a NSs deletion mutant (NSsdel), we infected primary human peripheral blood mononuclear cells (PBMCs) with different multiplicities of infection for different periods of time. By measuring the inflammatory response by ELISA, our studies demonstrate that MP-12-induced IFN-$\alpha$ responses are attenuated compared to NSsdel. Surprisingly, isolated plasmacytoid dendritic cells (pDCs) showed a dose dependent type-I IFN response to RVFV MP-12, suggesting a mechanism by which these cells are able to overcome NSs-induced suppression. Studies with inactivated viruses suggest that viral replication may be required for an IFN$\alpha$ response. Viral plaque assays as well as quantitative polymerase chain reaction studies show that both viral strains can replicate easily in the mixed PBMC population, although NSsdel does not replicate as well as MP-12. MP-12 viral burden was similar between PBMC and pDC-depleted PBMC, but reduced in isolated pDCs. Diminished viral burden in pDC may have resulted from the robust IFN-$\alpha$ responses documented. Analysis of viral copy number by PCR indicates that the RVFV genome may be able to replicate equally well in all PBMC cell types, but different cell types may have mechanisms by which they disrupt the formation and/or egress of infectious virions.

## 294

### GEOGRAPHIC AND CLIMATOLOGIC RISK FACTORS ASSOCIATED WITH THE 2006-2007 KENYA RIFT VALLEY FEVER OUTBREAK: A POPULATION-BASED MULTIVARIABLE MODEL

**Allen Hightower**[1], Carl Kinkade[2], Patrick Nguku[3], Tom Ksiazek[4], S. K. Sharif[3], Samuel Amwayi[3], David Schabel[5], Daniel Feikin[1], Maurice Ombok[1], Kariuki Njenga[1], Robert Breiman[1]

*[1]CDC - Kenya, Nairobi, Kenya, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Ministry of Public Health, Nairobi, Kenya, [4]University of Texas, Medical Branch, Galveston, TX, United States, [5]US Army Medical Research Unit – Kenya, Nairobi, Kenya*

Prediction of epidemic RVF has been the subject of many large-scale modeling efforts. Given its occurrence as an intersection of climatic conditions, the ecological niche of the vectors, and nearby susceptible populations, modeling on national or more local scales, where richer datasets exist, should yield useful information. 215 of 340 (63%) laboratory-confirmed and probable cases of RVF in the Kenya outbreak of 2006-2007 were geocoded. Kenya was divided into a grid of 46,200 3.5 km sq cells to provide a basis for analyses. The number of cases, estimated population, soil type and related factors, land form, regional slope, land cover, ecological zones, elevation, rainfall, and vegetation indices for each 10 day period of the outbreak were determined for each cell in the grid. A multivariable poisson regression model using population as an offset was developed using the data from all cells. Several specific soil types were related to RVF disease: solonchaks (RR=123.5, 95%CI=71.4, 210.3), planosols (RR=24.4, 95% CI=16.4, 36.6), calcisols (RR=14.7, 95% CI=7.1, 28.2), and solonetz (13.6, 95% CI = 9.0, 20.7). Rainfall in the first 10 days of November 2006 (RR per cm: 1.16, 95% CI=1.13, 1.19), a dense bush land cover (RR=7.1, 95% CI= 5.1, 9.9) and a "flat" regional slope (vs hilly, others) (RR=5.4, 95% CI=3.5, 8.7) were also independently associated with RVF incidence. Baseline incidence in the absence of these factors was less than 1 per million person years. In conclusion, all significant soil types have substrata that retain moisture better than other soil types. Solonchak soil types found in Baringo dry out to become solonetz types found in NE province, providing a potential linkage to the case occurrence in these two sites. Other factors, such as flatness and bush cover may have contributed to improved ecology for mosquito breeding and survival. This statistical model may provide useful information for refining prediction models by identifying factors to consider in addition to rainfall forecasts and populations at risk.

## 295

### A NOVEL ANTIMALARIAL CHEMOTYPE WITH EFFICACY AGAINST BOTH EXOERYTHROCYTIC AND ERYTHROCYTIC STAGES

**Jane X. Kelly**[1], Michael O'Neil[2], Victor Melendez[2], Erin Harris[2], Arba Ager[3], Martin Smilkstein[4], Isaac Forquer[1], Rosie Dodean[1], Rolf Winter[1], Dave Hinrichs[1], Mike Riscoe[1]

*[1]Portland VA Medical Center, Portland, OR, United States, [2]Walter Reed Army Institute of Research, Division of Experimental Therapeutics, Silver Spring, MD, United States, [3]University of Miami, School of Medicine, Miami, FL, United States, [4]Oregon Translational Research and Drug Development Institute, Portland, OR, United States*

Our development of antimalarial compounds with an acridone core has led to the identification of several distinct chemotypes, determined by the choice of functional groups. In addition to previously-described potent respiratory inhibitors and dual function chemotypes, we now report another functionally-distinct chemotype which displays efficacy against sporozoite-induced *Plasmodium* infection in addition to *in vitro* efficacy against the blood stage. Efficacy of an initial lead compound (T2) has been demonstrated in the following systems: (1) Prevention of *in vivo P. yoelii* sporozoite-induced blood stage infection in mice; (2) Prevention of *in vitro P. berghei* sporozoite-induced development in mouse hepatocytes; (3) Low nanomolar inhibition of *in vitro P. falciparum* blood stage growth; and (4) Curative efficacy after oral administration in models of murine malaria. As with our other acridone chemotypes, initial screening reveals no concerning evidence of mammalian cytotoxicity, genotoxicity, or animal toxicity. Initial mechanistic studies suggest a complex mode of action, including but not limited to inhibition of the parasite electron transport chain. Given the paucity of water-soluble drug candidates with exoerythrocytic efficacy, further investigation of this chemotype certainly is warranted. Details of the design, chemistry, and preliminary studies of safety, metabolism and mechanism of action will be presented.

## 296

### NEW INSIGHTS INTO MECHANISM OF HEMOLYTIC TOXICITY AND ANTIMALARIAL EFFICACY OF 8-AMINOQUINOLINES: EVALUATION OF STEREOSELECTIVE PROFILES

**Babu L. Tekwani**[1], Rajnish Sahu[2], N. P. Dhammika Nanayakkara[2], Larry A. Walker[1]

*[1]National Center for Natural Products Research and Department of Pharmacology, School of Pharmacy, University of Mississippi, University, MS, United States, [2]National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS, United States*

The effect of isomerism on biological activity and toxicity of drugs is well documented. When drugs are introduced in the body, physiological processes show a high degree of chiral distinction. Differential interactions of isomers with chiral targets are responsible for this discrimination. Most of the antimalarial drugs in clinical use are chiral but are used as racemates. For antimalarials namely primaquine, halofantrine and chloroquine, stereoselectivity has been noted in their ability to cause toxicity. Recent studies at our lab have established stereoselective toxicity and efficacy profile of NPC1161C, (±)-8-[(4-amino-1- 4 methylbutyl) amino]-6-methoxy-4-methyl-5-[3,4-dichlorophenoxy] quinoline succinate. The study was further extended to include isomers of primaquine to determine if other 8-AQs with the same asymmetric center display similar properties. The analogs were separated into individual isomers by fractional crystallization of diastereomeric salts formed with chiral organic acids. The method provided highly purified preparations of individual isomers as confirmed by chiral HPLC. The isomers along with racemates were evaluated *in vitro* through a metabolism-linked methemoglobin toxicity assay and also *in vivo* in *Plasmodium berghei*- mouse malaria models. Better antimalarial efficacy of NPC1161B (l-isomer) than NPC1161A (d-enantiomer) was confirmed. However, in the case of PQ, the d-isomer was more toxic and also more efficacious *in vivo* in *P. berghei* mouse malaria models. The NPC1161C isomers exhibited distinct stereoselective effects *in vitro* in methemoglobin toxicity assays while no significant difference was noticed between the PQ isomers. Differential pharmacokinetic and pharmacodynamic characteristics of isomers of PQ and NPC1161C may contribute to these differences. The studies should be useful in understanding the mechanism of stereoselective toxicity/efficacy profiles and shall aid in efforts to improve the therapeutic index of 8-AQs. Supported by U.S. Army Medical Research and Materiel Command and Gates Foundation.

## 297

### AN *IN VIVO* GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD)-DEFICIENT MOUSE MODEL TO PREDICT THE HEMOLYTIC TOXICITY OF PRIMAQUINE

**Prabhati Ray**[1], Peng Zhang[1], Xiugong Gao[1], Hiroshi Ishida[1], Aileen Chua[1], Jack Amnuaysirikul[1], Peter J. Weina[1], Max Grogl[1], Colin Ohrt[1], Aruna Sampath[2], Alan J. Magill[1]

*[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]University of Mississippi, University, MS, United States*

G6PD deficiency (G6PDD) is one of the most common human genetic polymorphism. G6PDD results in an impaired ability of the red blood cells (RBCs) to deal with oxidative stress and can cause hemolytic anemia, usually after exposure to certain medications, food, or even infections. Antimalarial drugs that can cause acute hemolysis in people with G6PDD include 8-aminoquinolines (8-AQs) such as primaquine (PQ). PQ remains the standard by which other 8-AQs are compared. To utilize the full benefit of the 8-AQ group of drugs, a thorough investigation of their hemolytic activity in G6PD deficient RBCs is fully warranted. In this study it was tested whether PQ administration could cause acute hemolysis in a G6PDD mouse model. Mice displaying a degree of G6PDD similar to that observed in the African-type human deficiency (~15% of normal) were used. PQ, 75mg/kg, was given in one day intraperitoneally in single or split doses. Blood was collected by tail nicking. Hemolytic parameters to include RBC count, hemoglobin (Hb), hematocrit, haptaglobin (Hp), reticulocytes, Heinz body, glutathione (reduced and total) and lactate dehydrogenase (LDH) were measured at day 4 and 7 after PQ. RBC count, Hb and hematocrit were similar in G6PDD and wild type animals. Reticulocytes, Heinz body formation and LDH were increased, whereas blood Hp and reduced glutathione (GSH) were decreased significantly in G6PDD mice compared to wild type mice. These results indicate that the G6PDD mouse used here may be a predictive model for studying the hemolytic toxicity of PQ.

## 298

### MIRINCAMYCIN: REASSESSMENT OF A PROMISING ANTI-MALARIAL AGENT WITH POTENTIAL IN A *PLASMODIUM CYNOMOLGI* RELAPSING MALARIA MONKEY MODEL

**Susan D. Fracisco**[1], Montip Gettayacamin[2], Victor Melendez[1], Qigui Li[1], Pisit Khemawoot[2], Bryan L. Smith[1], David Saunders[2], Kent Bennett[1], William McCalmont[1], Charlotte Lanteri[1], Geoffrey Dow[1], Yarrow Rothstein[1], Carl Craft[3], Paktiya Teja-isavadharm[2], Imerbsin Rawiwan[2], Alan Magill[1], A.J. Lin[1], Ian Bathurst[3], Richard Westerman[4], Colin Ohrt[1]

*[1]WRAIR Experimental Therapeutics, Silver Spring, MD, United States, [2]Armed Forces Research Institute of the Medical Sciences, Bangkok, Thailand, [3]Medicines for Malaria Venture, Geneva, Switzerland, [4]MALDEVCO, Kalamazoo, MI, United States*

Historically, mirincamycin, a clindamycin analog, produced cures in a relapsing monkey model. The historical experiments were limited by an incomplete understanding of the drug's pharmacodynamic/pharmacokinetic (PK/PD) relationship. Extensive PK sampling was not done and the analytic methodology was insufficient to detect the drug at low enough levels to allow full characterization of drug exposure. As part of a reassessment of mirincamycin's potential for antihypnozoite activity, we conducted a series of efficacy and PK experiments in mice and monkeys. Dose- response for cis- and trans-mirincamycin was characterized in a causal prophylactic *Plasmodium berghei*-infected mouse model using a 3 day dosing schedule. Rodent PK was assessed by single dose oral and SC plasma levels of each isomer at 8 time points. Bioavailability (BA) and PK analyses for each isomer in Rhesus (*Macaca mulatta*), Indian-origin, were determined in a single dose parallel design. Plasma was sampled from 0-168 hours and measured via LC-MS. Data from both PK studies were analyzed using non-linear regression software. Radical curative assessment of each isomer was tested following treatment of sporozoite induced blood stage infections with chloroquine and mirincamycin using the relapsing parasite *Plasmodium cynomolgi* (Bastionelli) in Rhesus monkeys. SC and oral ED90 of both isomers in mice were from 2-3 mg/kg administered on days -1, 0, 1 with sporozoite inoculations on day 0. For both isomers in Rhesus, BA was estimated at 12%, with an elimination t½ of 9-12 hours. Drug levels in an assay sensitive to 3 ng/ml did not detect drug by day 7. Mouse experiments produced similar results. No toxicity was seen except for transient, self-limited soft stools in the treated monkeys. Data from an ongoing confirmation experiment of the radical curative activity of these compounds in a relapsing malaria model will also be presented. In conclusion, rhesus and mouse PK experiments strongly suggest the prior reported radical curative activity of mirincamycin was not a false positive result due to prolonged suppressive activity of a blood stage-active drug. The efficacy and PK/PD will provide more definitive evidence of true antihypnozoite activity. These data, in part, will allow us to determine whether the antimalarial activity of mirincamycin and its analogs can be utilized without the unwanted side effects associated with this antibiotic class.

## 299

### UNDERSTANDING THE ANTIMALARIAL ACTION OF THE HIV PROTEASE INHIBITORS

**Tina S. Skinner-Adams**[1], Katherine T. Andrews[2], Donald L. Gardiner[1], James S. McCarthy[1]

*[1]Queensland Institute of Medical Research, Brisbane, Australia, [2]Eskitis Institute for Cell and Molecular Therapies, Griffith University, Brisbane, Australia*

Malaria remains a significant cause of morbidity and mortality worldwide with an estimated 1 million people dying from this disease every year. Drug resistance is widespread, and with a safe and effective vaccine still many years away, new chemotherapeutic agents are required to ensure that cheap and effective treatment is widely available. We have demonstrated that some antiretroviral protease inhibitors (APIs) kill malaria parasites *in vitro* and *in vivo* at clinically relevant concentrations, a finding whose clinical significance we are now determining in multi-centre clinical trial in malaria endemic regions of Africa. While our *in vitro* isobologram data demonstrate that the antimalarial activity of the API's is likely to benefit those people who are co-infected with HIV and malaria, they are not appropriate for first-line antimalarial agents in their own right. Nevertheless, the antimalarial target of these anti-HIV drugs represents a completely new approach to the development of malaria parasite-specific therapies. More recent data now demonstrate that APIs have an activity against a broad range of parasite developmental stages, and that these drugs kill malaria parasites by acting on an uncharacterized non-digestive vacuole plasmepsin that is yet to be exploited as a potential target for antimalarial drug development.

## 300

### EFFICACY OF PYRONARIDINE/ARTESUNATE IN CLINICAL TRIALS IN PATIENTS WITH UNCOMPLICATED ACUTE *PLASMODIUM FALCIPARUM* OR *PLASMODIUM VIVAX* MALARIA: RESULTS OF AN INTEGRATED ANALYSIS

**Stephan Duparc**[1], Isabelle Borghini-Fuhrer[1], J. Carl Craft[2], Sarah Arbe-Barnes[3], Robert M. Miller[3], Chang-Sik Shin[4], Lawrence Flenckenstein[5]

*[1]Medicines for Malaria Venture, Geneva, Switzerland, [2]Former Medicines for Malaria Venture, Geneva, Switzerland, [3]Fulcrum Pharma Developments Ltd, Hemel Hempstead, United Kingdom, [4]Shin Poong Pharmaceuticals, Seoul, Republic of Korea, [5]University of Iowa, Iowa City, IA, United States*

Pyronaridine/artesunate (Pyramax®/PA) is a novel and promising artemisinin-based combination therapy (ACT) being developed in a Private-Public-Partnership between Shin Poong Pharm, Medicines for Malaria Venture and University of Iowa. This fixed-dose combination of pyronaridine tetraphosphate and artesunate in a 3:1 ratio, is being developed as an oral treatment OD for 3 days, for uncomplicated *Plasmodium falciparum* (*Pf*) and for blood stage *P.vivax* (*Pv*) malaria in adult and pediatric patients. The clinical development program is now completed and the registration dossier should be submitted to stringent regulatory authorities by end of 2009 then to National Authorities in Asia and Africa. The Phase III program included 2 trials on *Pf* comparing PA to artemether/lumefantrine (AL) and to mefloquine + artesunate; 1 trial in young children and infants (body weight: 5-25 kg) using the pediatric granule formulation of PA versus AL; and 1 trial for *Pv* malaria comparing PA to chloroquine. Three of these phase III studies were presented at ASTMH 2008 in New Orleans and one at ECTM 2009 in Verona. Integrated analyses of efficacy (ISE) and safety (ISS) were performed and the outputs and conclusions for ISE are discussed in this presentation. All Phase III studies met their primary efficacy endpoint, demonstrating non-inferiority of PA to the comparators, with cure rates higher than 95%. The primary efficacy variable was also analyzed by region, age-group, gender, baseline parasitemia and previous malaria episodes and were in line with results obtained in the total population studied, though a difference between Africa and Asia was observed for parasite clearance time. This ISE demonstrated high levels of efficacy of PA in *Pf* and *Pv* adults and children malaria patients. PA showed high cure rates, similar to those of the current standard of care therapies. PA provides rapid clearance of parasitemia and most malaria-related symptoms, coupled with prevention of recrudescence. The relatively long half-life of pyronaridine could explain the observed prophylactic effect up to day 42. PA is a promising new ACT for the treatment of acute uncomplicated *Pf* or *Pv* malaria in adults and children from 5kg.

## 301

### SAFETY, EFFICACY AND PHARMACOKINETIC COMPARISON OF FIXED-DOSE ARTESUNATE-AMODIAQUINE "ASAQ" WITH NON-FIXED COMBINATION OF AS AND AQ AMONG KENYAN ADULTS

**Bernhards Ogutu**[1], Elizabeth Juma[1], Vincent Jullien[2], Gwenaelle Carn[3], Jean-René Kiechel[3]

*[1]Kenya Medical Research Institute (KEMRI), Nairobi, Kenya, [2]Cardinal Health Systems, Paris, France, [3]Drugs for Neglected Diseases initiative (DNDi), Geneva, Switzerland*

WHO guidelines recommend artemisin-based combination therapies (ACTs) to treat uncomplicated *Plasmodium falciparum* malaria; and fixed-dose combinations (FDCs) could improve compliance, accessibility, and protect the one effective class of antimalarial medicines. However, key efficacy, effectiveness, tolerability, and PK characteristics should be shown to be similar to a combination of separate products used together. This randomized clinical study was conducted in Kenyan adults at one center in Kusumu, a highly endemic region, and consisted of a 3-day treatment period and 25-day follow-up. Patients (54 were randomized into the study) received either 2 tablets of FDC 100mg/270mg AS/AQ respectively, or 4 tablets of 50 mg AS + 153 mg AQ. The primary objective was to compare pharmacokinetic parameters of FDC AS/AQ with those of the non-fixed combination. Efficacy and tolerability, including ECG, were also assessed. Artesunate and dihydroartemisinin were rapidly eliminated, as is typically observed. The elimination half-life of DeAQ, the main metabolite of amodiaquine, also had values comparable to literature. Pharmacokinetics were similar in both arms, with slightly higher AUCs corresponding differences seen in the administered dose. Both treatments were efficacious, with only two late treatment failures (re-infections); final analysis is in progress. In ECG, the AS/AQ FDC did not cause a significant prolongation of the QTc interval as compared with non-fixed AS+AQ. The changes in both patient groups were most likely related to heart rate changes and fever decrease due to treament. No other significant cardiac effects were seen. Preliminary safety analysis shows both treatments to be well-tolerated. In conclusion, this Phase 2 study provides important data about ASAQ in an adult population of a highly endemic, East African region, and confirms the good tolerability and efficacy of this FDC. PK analysis has provided important data on this FDC in adults, complementing the earlier pivotal study of FDC in Burkina Faso children.

## 302

### RELATIONSHIP BETWEEN BITE-TO-HOSPITAL TIME AND MORBIDITY IN VICTIMS OF CARPET VIPER BITE IN NIGERIA

**Oluwagbenga P. Ogunfowokan**[1], Jacob A. Dawam[2], Livinus L. Odor[2]

*[1]National Hospital Abuja, Abuja, Nigeria, [2]Jos University Teaching Hospital, Jos, Nigeria*

Envenomation resulting from snake bites is an important public health hazard in tropical and subtropical countries. The aim of our study was to find out the relationship between bite-to-hospital time and morbidity in patients, bitten by carpet viper ( *Echis Ocellatus*). A prospective study was conducted in a rural community in Northcentral Nigeria from December 2007 to February 2008. A morbidity score was computed to assess the extent of morbidity in each patient from the time of admission to discharge from hospital. A score of 1 was given to each objective and

verifiable sign. The same sign occurring in more than one area of the body attracted additional unit score each. The signs of morbidity scored were edema, tenderness, blister, ulcer, need for blood transfusion, unconsciousness, hypotension, convulsion, angioedema, local bleeding, systemic bleeding, prolonged whole blood clotting time on presentation, length of hospital stay, need for disarticulation, and need for skin graft. Each day on admission attracted a unit score. Bite-to-hospital time of 233 subjects with known morbidity outcomes was obtained. Relationship between bite-to-hospital time and morbidity was determined. The median bite-to-hospital time was 5 hours with range 0.5 - 216 hours. Major causes of morbidity were edema, tenderness and bleeding accounting for 212 (91.0%; 95% CI 86.6 - 94.3%), 201 (86.3%; 95% CI 81.2 - 90.4%), and 75 (32.2%; 95% CI 26.2 - 38.6%) respectively. The mean morbidity score was 8 ± 4. For every unit increase in log bite-to-hospital time, the morbidity score increased by 1.9 in a linear fashion ($p < 0.001$). There was a linear trend in relationship between increasing bite-to-hospital time group and blood incoagulability at presentation (p for linear trend = 0.02). In conclusion, Morbidity caused by carpet viper bite is high in Nigeria and correlates with increasing bite-to-hospital time.

## 303

### EFFECT OF THE A-FORM OF G6PD DEFICIENCY ON MATERNAL *PLASMODIUM FALCIPARUM* PARASITEMIA AND PREGNANCY OUTCOMES

**Ella T. Nkhoma**[1], Jianbing Mu[2], Michael A. Krause[2], Seidina A. Diakite[3], Linda Kalilani[4], Stephen J. Rogerson[5], Rick M. Fairhurst[2], Steven R. Meshnick[1]

*[1]University of North Carolina-Chapel Hill, Chapel Hill, NC, United States, [2]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, [3]Department of Immunogenetics, University of Bamako, Bamako, Mali, [4]College of Medicine, University of Malawi, Blantyre, Malawi, [5]Department of Medicine, University of Melbourne, Parkville, Australia*

The objective of this study was to determine the effect of G6PD*A- allele carriage on maternal parasitemia and pregnancy outcomes. Between 2005 and 2006, we followed pregnant women attending two antenatal care clinics in southern Malawi from the second trimester of gestation until delivery. We conducted a case-cohort study by sampling all cases of placental parasitemia and sampling a 35% randomly selected subcohort of all women followed until delivery. The G6PD*A- allele status of both groups of pregnant women was assessed. There were 46 women with placental parasitemia and 294 women were included in the subcohort. The prevalence of placental parasitemia and G6PD*A- carriage in the subcohort were 16% and 28%, respectively. Primigravidae carrying G6PD*A- were as likely to have placental parasitemia as primigravidae not carrying G6PD*A- (Prevalence Ratio (PR) =1.0, 95% confidence interval (CI): 0.7, 1.3). Among multigravidae, G6PD*A- carriers were 0.9 (95% CI: 0.8, 1.0) times as likely to have placental parasitemia as G6PD*A- non-carriers. Further, their placental parasite density was on average 0.22 (95% CI: 0.08, 0.56) times that of G6PD*A- non-carriers. Among primigravidae, G6PD*A- carriers had 1.7 (95% CI: 1.0, 2.9) times the average risk of maternal anemia over follow-up when compared to G6PD*A- carriers. This was not observed for multigravidae. Across gravidities, G6PD*A- was associated with an increased risk of low birth weight (LBW, PR=2.5, 95% CI: 1.2, 5.2). Among daughters, G6PD*A- carriage by the mother was associated with a 286g reduction in mean birth weight ($p = 0.02$). A reduction in mean birth weight was not observed among sons. In conclusion, the results of this study suggest a lack of a protective effect for G6PD*A- carriage on parasitemia and anemia risk. However, among multigravidae, G6PD*A- carriage may protect against high parasite density. We also found an increased risk of LBW due to maternal G6PD*A- carriage. More studies will need to be conducted to understand the role of G6PD*A- carriage during pregnancy in malaria-endemic areas.

## 304

### A COMPARISON OF IRON AND FOLATE WITH FOLATE ALONE IN HEMATOLOGIC RECOVERY OF CHILDREN TREATED FOR ACUTE MALARIA

Samuel N. Gara[1], Aboi J. Madaki[1], **Tom D. Thacher**[2]

*[1]Jos University Teaching Hospital, Jos, Nigeria, [2]Mayo Clinic, Rochester, MN, United States*

Concern has been raised that iron treatment in acute malaria may worsen the severity of malaria and interfere with recovery. We conducted a randomised controlled trial to compare the effect of iron and folate with folate alone on hematologic recovery in children treated for acute malaria. We randomized consecutive Nigerian children, 6-60 months of age, who presented with smear-positive malaria and anemia (hematocrit<33%) to receive iron as ferric ammonium citrate (2 mg/kg/day elemental iron) plus folate (5 mg/day) or folate alone, in addition to antimalarial drug treatment. Hematocrit was measured at baseline and four weeks after treatment. Of 417 Nigerian children who presented with fever, 176 (42%) had malarial parisitemia. Of these, 82 (47%) had a hematocrit<33% and were randomized to treatment. Mean (±SD) hematocrit at baseline was 28.5±2.9%. One child in the folate group required hospitalization and transfusion, and one child in each group failed to return for follow up. At four weeks, the mean hematocrit increased by 2.5±1.6% in the iron plus folate group and 1.4±1.0% in the folate alone group (P=0.001). In multivariate regression, the baseline hematocrit, iron supplementation, and child's weight for height were significant predictors of final hematocrit. The effect of iron was not significantly modified by the baseline hematocrit, weekly meat intake, nutritional status, mother's education, sex, or age of the child. In conclusion, four weeks of supplementation with iron and folate provides greater improvement in hematocrit than folate alone in children with malarial anemia.

## 305

### IMPROVED MALARIA CASE MANAGEMENT FOLLOWING DISTRICT BASED INTEGRATED TEAM TRAINING AND SUPPORT SUPERVISION OF HEALTH CARE WORKERS IN UGANDA

**Umaru B. Ssekabira**[1], Dan Senjovu Kaggwa[1], Alex Ojaku[1], Patrick Eyul[1], Adoke Yeka[2], Priscilla Aquilla Omwangangye[3], Allen Namagembe[1], Samuel Ocan[1], John Bosco Rwakimari[4], Lydia Mpanga Sebuyira[3], Marcia R. Weaver[5]

*[1]Joint Uganda Malaria Training Programme, Kampala, Uganda, [2]Infectious Diseases Research Collaboration, Kampala, Uganda, [3]Infectious Diseases Institute of Makerere University, Kampala, Uganda, [4]Uganda Ministry of Health, Malaria Control Programme, Kampala, Uganda, [5]University of Washington, Seattle, WA, United States*

Malaria case management in Africa is characterized by presumptive treatment and substantial overtreatment. Health workers often do not manage patients appropriately; malaria laboratory tests are rarely requested. When tests are performed, health workers prescribe antimalarials despite negative test results. Training is necessary to improve malaria case management, but it's difficult to scale up using national level training. We tested the effectiveness of the integrated team-based training program delivered by district trainers at 2 government health centers in Uganda. Clinicians, laboratory staff, and records clerks were trained. Training covered general aspects of malaria, clinical evaluation and treatment of febrile patients, malaria microscopy skills, and medical record keeping. Two support supervision visits were made 6 and 12 weeks after training. Performance of clinicians (n=22) and laboratory staff (n=4) was assessed at baseline and during the visits. Outcomes were measured using a surveillance system that captured data on outpatient consultations and laboratory results. Data collected before (n=64 days) and after (n=87 days) training were compared. Diagnostic accuracy of field microscopy before and after training was compared using expert microscopy as the gold standard. The proportion of clinicians who; took

proper history increased from 9% to 59.1%, examined patients properly increased from 0% to 27.3%, diagnosed correctly increased from 35.2% to 55%, counseled patients adequately increased from 0% to 66.7%. The proportion of suspected malaria patients referred for microscopy increased from 30% to 65.5% (*P*=0.025) and the proportion with negative blood smears prescribed antimalarials decreased from 76.8% to 51.2% (*P*=0.001) in those < 5 years. The sensitivity increased from 27.5% to 97% and specificity rose from 63% to 85%. The percentage agreement (Kappa) between laboratory staff and expert microscopists increased from 0.02 to 0.94. The intervention improved key indicators of malaria case management and reduced unnecessary antimalarial treatments.

## 306

### DISPARITIES EXIST IN THE AVAILABILITY OF MALARIA TREATMENT IN THE U.S.

**Kelly A. Bear**, Amanda Higginson, Patrick Hickey

*Uniformed Services University of the Health Sciences, Bethesda, MD, United States*

The Maryland suburbs of Washington, D.C. are a major center of imported malaria. Correlation of Census Bureau data for sub-Saharan African (SSA) ethnicity and mapping of case residence demonstrates that geographic risk zones are identifiable. Low usage of prophylaxis is a known risk behavior of immigrant groups, but the impact on community availability of therapy is unknown. This study reports the availability of anti-malarial medication (AMM) in areas with differing risk and socio-economic profiles. A blinded telephone survey of 43 pharmacies in 10 zip codes was conducted to determine the availability of AMM. Selected zip codes were aggregated based on historical risk of malaria and demographics. The survey recorded which AMM were kept in-stock or the time needed to obtain them. Comparisons of medication availability were made across risk groups using a chi-square test. Pharmacies in high income zip codes, with lower SSA population density and low malaria case rates, were more likely to stock first line therapy medications, atovoquone-proguanil or quinine, (p=0.04) than those with lower income, high SSA density, and higher rates of reported malaria (p=0.08) as compared to low income, low malaria, low SSA zip codes. Only 4 (9.7%) pharmacies stocked quinine, many believing that it was no longer FDA approved- a mistaken interpretation of a warning related to its use in the treatment of restless leg syndrome. In conclusion, the availability of anti-malarial medications was more closely associated with wealth and low minority population than risk, as indicated by prior cases and size of the SSA population. We hypothesize this difference is due to differing rates of prophylaxis usage. We discovered that most pharmacies in the area are no longer stocking quinine. These findings also have implications for the U.S. role-out of artemether-lumefantrine, which has no role in prophylaxis. Clinics and emergency departments should be aware of the limited availability of first line therapy medications when considering outpatient therapy.

## 307

### PROSPECTIVE STUDY ON CO-INFECTION OF COMMON VIRAL RESPIRATORY PATHOGENS AND MALARIA IN YOUNG CHILDREN IN PAPUA NEW GUINEA

**Patricia Rarau**, Mary Salib, Doris Manong, Brenda Wingi, Jennifer Igu, Ivo Mueller, Nicolas Senn

*Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea*

In Papua New Guinea (PNG), malaria and lower respiratory tract infections (LRTI) are leading causes of morbidity and mortality in children under 5 years of age. The introduction of rapid diagnostic tests (RDT) for malaria has greatly improved the clinical management of patients presenting with fever. However, a significant proportion of patients presenting with a positive test for malaria also present features of LRTI such as cough and fast breathing. To investigate such co-infections, children presenting with a positive diagnosis for malaria and criteria for LRTI during the PNG Intermittent Preventive Treatment in infants (IPTi) trial, were tested for the most frequent viral respiratory pathogens (Respiratory Syncytial Virus (RSV), Influenza A & B and Adenovirus) using rapid tests performed on nasal swabs. Initial results indicate the presence of a viral infection in 35/52 (67%) of children aged 4-27 months with a diagnosis of malaria plus signs/symptoms of LRTI. Viral co-infection was most frequent in children 12-24 months of age (70%) followed by children <12 months (54%) and children >24 months (50%). Influenza B accounted for 42% (13/31) of all positive cases, followed by Influenza A with 26% (8/31) and RSV with 19% (6/31). Lastly 13% (4/31) for the mixed infections, two cases of adenovirus with Influenza B, 1 case of Influenza A with RSV and another case of Influenza A with Influenza B. Complete results will be presented and highlight the importance of viral co-infections in children with a positive diagnosis of malaria. Clinical management of patients presenting with a positive test for malaria and signs/symptoms of low respiratory tract infections thus needs to consider viral co-pathogen.

## 308

### INVESTIGATION OF CONTACTS OF A SOUTH AFRICAN TRAVELER WITH INITIALLY SUSPECTED ARENAVIRUS INFECTION, RIO DE JANEIRO, BRAZIL, 2008

**Priscilleyne O. Reis**[1], Dalva M. Assis[1], Alessandra V. Cardoso[2], Cecília C. Nicolai[3], Guida Silva[4], Ligia M. Costa[5], Elba R. Lemos[6], Márcio H. Garcia[7], Wildo N. Araújo[1], Eduardo H. Carmo[8]

*[1]Brazilian Field Epidemiology Training Program (EPISUS), Secretariat of Health Surveillance, Ministry of Health, Brasília, Brazil, [2]Respiratory an Vaccine Preventable Disease Branch, Ministry of Health, Brasília, Brazil, [3]Municipal Secretariat of Health, Rio de Janeiro, Brazil, [4]State Secretariat of Health and Civil Defense, Rio de Janeiro, Brazil, [5]Directorate of Public Health Laboratories, Ministry of Health, Brasília, Brazil, [6]Department of Virology, Oswaldo Cruz Institute, Ministry of Health, Brasília, Brazil, [7]Center for Strategic Information in Health Surveillance, Ministry of Health, Brasília, Brazil, [8]Department of Epidemiological Surveillance, Secretariat of Health Surveillance, Ministry of Health, Brasília, Brazil*

Introduction: In 2008, Brazil notified WHO of an international public health emergency when a South African traveler was hospitalized with suspected arenavirus infection in Rio de Janeiro, following possible recent contact with cases of arenavirus from a highly lethal outbreak in South Africa months earlier. The traveler had symptom onset 48 hours after landing in Rio, with rapid progression; death occurred seven days later. The laboratory-confirmed diagnosis was rickettsiosis; infection probably occurred in South Africa before travel. Until arenavirus infection was ruled out, concern about respiratory transmission of a high fatality disease led us to categorize and monitor potential contacts in accordance with the nature of the contact. Objectives: describe the classification and monitoring of contacts of the ill traveler. Methods: We applied the following categories of exposure to patient contacts: CI - persons with cutaneous or mucosal exposure to patient's blood or secretions were monitored for symptoms twice daily by phone; CII - persons with frequent direct contact with patient, oriented as those of CI and requested to self monitor for symptoms; CIII - persons who stayed at same location as patient, oriented about symptoms based on their own request. Results: Of 101 persons with contact at hospitals, the patient's work site, and the patient's hotel, 64(63%) were evaluated: 25 were classified as CI, 34 as CII, and 5 as CIII. We were unable to reach 24 potential contacts. One contact presented symptoms, received antiviral medication, was monitored and recovered without incident. Conclusions: Categorization and monitoring of contacts was useful in testing the application of the International Sanitary Regulation. Monitoring was effective in ruling out transmission of illness to contacts before definitive diagnosis was available and reduced anxiety among the contacts. We recommend enhancement of protocols for control of severe respiratory diseases of unknown etiology and training local and state public health officials in responding to similar events.

## 309

### A RAT MODEL OF INTRACEREBRAL INFECTION WITH *TAENIA CRASSICEPS* FOR THE STUDY OF INFLAMMATION ASSOCIATED WITH ANTHELMINTIC THERAPY IN NEUROCYSTICERCOSIS

**Siddhartha Mahanty**, Abby L. Berns, Erick Scott, Martin Lizak, Theodore Nash

*National Institutes of Health, Rockville, MD, United States*

Neurocysticercosis (NCC), infection of the larval form of Taenia solium (Ts) is a leading cause of adult-onset epilepsy in tropical regions. Treatment of NCC with conventional anthelmintics is frequently associated with exacerbation of neurological symptoms. Inflammation resulting from host immune reactions to the dying parasites has been implicated in the pathogenesis of post-treatment reactions. To date, rodents models of neurocysticercosis differ from human infections in several fundamental ways, making them unsuitable for the study of such reactions. We have developed a model of intracerebral infection in rats using *Taenia crassiceps* (Tc), a tapeworm closely related to Ts. A single cultured Tc metacestode was implanted into the peri-hippocampal parenchyma of each rat using stereotactic surgery. We then assessed the growth of the cysts, inflammatory response, immunohistopathology, and response to treatment over time. MRI studies revealed growth of cysts with median volume expansion up to 147 fold (n=5) over 13 weeks. Despite this sizeable growth, no neurological symptoms were apparent in infected rats. Eight to ten weeks PI, rats were treated for 14 days with praziquantel (PZ) and the presence and degree of post inflammatory responses studied. In a majority, but not all of the infected rats, IgG antibody titers against the cyst antigens ranging (Range of ELISA end-point titers: 1:200 to 1:1600) were detected. Circulating Tc antigens (Ag) were detected in the serum of 66% of infected rats; post-treatment, Ag levels increased in 66% (4/6) rats during treatment with PZ, with all showing a decline in levels by the end of treatment. Brain histopathology revealed patchy inflammatory infiltrates that were comprised of macrophages, eosinophils and lymphocytes surrounding cysts in untreated and PZ-treated infected rats. Ongoing studies will phenotype infiltrating cells surrounding the implanted cysts by immunohistochemistry and flow cytometry, and delineate the regulation of genes associated with inflammatory responses. Inflammatory responses that follow treatment with other anthelmintics, such as albendazole, are also being investigated using this model. This model provides a closer correspondence to human neuropathology in NCC than previously available animal models and has great potential for investigation of anti-inflammatory therapies in controlling post treatment reactions to anthelmintics in NCC.

## 310

### A MULTI-ANTIGEN PRINT IMMUNOASSAY (MAPIA) FOR DETECTION OF *TAENIA SOLIUM* CYSTICERCOSIS AND TAENIASIS ANTIBODIES

**Sukwan Handali**[1], Molly Klarman[2], Amanda N. Gaspard[3], John Noh[1], Yeuk-mui Lee[1], Silvia Rodriguez[4], Armando E. Gonzalez[5], Hector H. Garcia[6], Robert Gilman[7], Victor C. Tsang[8], Patricia P. Wilkins[1]

*[1]Centers for Disease Control and Prevention, Chamblee, GA, United States, [2]Rollins School of Public Health, Emory University, Atlanta, GA, United States, [3]Rollins School of Public Health, Emory University, Atlanta, GA, United States, [4]Cysticercosis Unit, Instituto de Ciencias Neurologicas, Lima, Peru, [5]School of Veterinarian Medicine, Universidad de San Marcos, Lima, Peru, [6]Department of Microbiology, Universidad Peruana Cayetano, Lima, Peru, [7]Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States, [8]Department of Biology, Georgia State University, Atlanta, GA, United States*

One of the most well characterized tests for diagnosis of neurocysticercosis is the enzyme-linked immunoelectrotransfer blot (EITB) developed at CDC that uses lentil-lectin purified glycoproteins (LLGP) extracted from *Taenia solium* cysticerci. Although this method is considered by many the gold standard for laboratory diagnosis, purification of the LLGP antigens has been difficult to standardize and the polyacrylamide gel system used for the immunoblot assay is not easily transferable to other laboratories. Therefore, over the last 10 years we systematically purified and cloned the diagnostic glycoproteins in the LLGP fraction. We found that the seven diagnostic proteins are members of 3 antigenic protein families: gp50, gp24, and the 8-kD family. Comparing the performance of these antigens, and 2 other antigens that are used to diagnose taeniasis, in classical formats such as in Western blot or in ELISA is not possible due to either similarity in molecular mass, *i.e.*, immunoblot, or because individual antigen responses can not be dissected, as is the case with ELISA. Multi-antigen printing immunoassay (MAPIA) or line immunoassay offers the opportunity to test the performance of all antigens in a single format. We developed MAPIA strips consisting of 8 cysticercosis and 2 taeniasis antigens and tested defined cysticercosis and taeniasis sera. Of 5 cysticercosis antigens, rT24H performed well in detecting cases with 2 or more viable cysts in the brain (sensitivity and specificity at 97% and 99.4%, respectively); combining cysticercosis antigens did not improve the sensitivity of the test, but decreased the specificity. All antigens performed similarly using sera from different clinical presentations of cysticercosis. Both of the taeniasis antigens (rES33 and rES38) performed well in diagnosing taeniasis (with both have sensitivity of 99.4%, and specificity of 93.9% and 94.5%, respectively). Some cross-reactivity against rES33 and rES38 was found, especially with sera from cases with *Schistosoma mansoni*. We conclude that MAPIA is a simple and effective tool to compare antibody responses to different cysticercosis and taeniasis antigens and may be a useful method for rapid detection of *T. solium* cases.

## 311

### EPILEPTIC SEIZURES IN POPULATION FROM ENDEMIC AND NON-ENDEMIC AREAS FOR CYSTICERCOSIS

Isidro Gonzalez[1], Jaime Miranda[2], Silvia Rodriguez[2], Candice Romero[2], Juan F Chiroque[2], Victor Vargas[1], Alfredo Cjuno[1], Javier A. Bustos[2], **Hector H. Garcia**[2]

*[1]Instituto de Ciencias Neurologicas, Lima, Peru, [2]Universidad Peruana Cayetano Heredia, Lima, Peru*

Up to 50% of late-onset epileptic seizures are associated with cysticercosis in areas where this condition is endemic. Participants, aged >30 years, were selected from a) residents and out-migrants from an endemic area and b) residents from a non-endemic area. A questionnaire aimed to identify history of epileptic seizures, followed by a neurological evaluation if positive, was performed. Serology for cysticercosis was assessed with EITB. The questionnaire was positive in 29.5% (233/789) and 23.5% (46/196) of endemic and non-endemic participants. The neurological examination confirmed epileptic seizures in 3.7% (29/789) and 2% (4/196) and active epilepsy in 2% (16/789) and 0.5% (1/196) of subjects from endemic and non-endemic areas, respectively. Cysticercosis seroprevalence in the endemic group was 14.6% (115/788) overall and 24% amongst those with epileptic seizures. In the non-endemic group, cysticercosis seroprevalence was 2% (4/198), mostly weak reactions and no association between serology and epileptic seizures was found in this group. In endemic populations for Cysticercosis, epileptic seizures are associated with higher seroprevalence and stronger serology reactions.

## 312

### EVALUATION OF NEW SEROLOGIC TECHNIQUES FOR THE DIAGNOSIS OF *STRONGYLOIDES STERCORALIS* INFECTIONS

**Alejandro J. Krolewiecki**[1], Roshan Ramanathan[2], Valeria Fink[3], Kimberly Won[4], Silvana Cajal[1], Marisa Juarez[1], Norma Acosta[1], Rogan Lee[5], Patrick Lammie[4], David Abraham[6], Thomas Nutman[2]

*[1]Instituto de Investigaciones en Enfermedades Tropicales, Oran, Argentina, [2]Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD, United States, [3]Fundacion Huesped, Buenos Aires, Argentina, [4]Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA, United States, [5]Institute of Clinical Pathology and Medical Research, Westmead Hospital, Westmead, Australia, [6]Department of Microbiology and Immunology, Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA, United States*

Human infections with *Strongyloides stercoralis* (Ss) often lack symptoms during the chronic stage. Diagnosis is problematic due to the requirement for labor intensive parasitologic techniques with suboptimal sensitivity. Serologic diagnosis based on recombinant antigens (RA) may provide a useful alternative. We evaluated 3 new serologic techniques for the diagnosis of Ss in comparison to a currently used ELISA assay and stool analysis. Patients from Argentina and Australia were evaluated with a combination of parasitologic techniques (Baermann`s, centrifugation, Agar plate, Harada Mori). Serologic assays based on the RA NIE were evaluated alone and in combination with a second RA (SsIR) in a Luciferase immunoprecipitation system (LIPS), and alone in an ELISA assay. An ELISA based on Ss crude extract (SsCE-ELISA) and stool analysis were used for comparison. Cutoff values were calculated using ROC curves. Spearman analysis between all techniques and performance including negative and positive predictive values (NPV and PPV) at different prevalences were calculated. The study included 262 samples (Argentina: 228, Australia: 23, US: 11). Cutoffs obtained from 85 stool positive samples and 10 stool negative samples from non-endemic regions were calculated at 29 (Luminescent Units)LU, 1203LU and 83U for NIE-LIPS, NIE-SsIR LIPS and NIE-ELISA respectively. Positive serologic responses were significantly associated with positive stool results (P<0.001). Sensitivity/specificity were best for SsCE-ELISA and NIE-LIPS techniques, 97/100 and 98/100 respectively. PPV was 100% for disease prevalences as low as 0.001% and NPV was >97% for SsCE-ELISA and >98% for NIE-LIPS at disease prevalences up to 50%. For all assays, having other coincident soil transmitted helminths did not alter the efficacy of the serological assays. In conclusion, NIE LIPS is a simplified and highly accurate serologic technique for the diagnosis of Ss infections, with high predictive values, comparable to SsCE-ELISA. Although not addressed directly in this study, species-specific antigens in a simplified format (as NIE-LIPS) provide additional accuracy and ease of performance in the serology-based diagnosis of Ss infections irrespective of infection prevalences.

## 313

### QUANTITATIVE PCR-BASED ASSESSMENT OF *ANGIOSTRONGYLUS CANTONENSIS* LARVAE BURDEN IN U.S. ENVIRONMENTAL SAMPLES

**Yvonne Qvarnstrom**[1], Henry Bishop[2], John Teem[3], Robert Hollingsworth[4], Sarah Y. Park[5], Arlene Buchholz[5], Alexandre J. da Silva[2]

*[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]CDC-NCZVED-Division of Parasitic Diseases, Atlanta, GA, United States, [3]Division of Aquaculture, Florida Department of Agriculture and Consumer Services, FL, United States, [4]United States Pacific Basin Agricultural Research Center, United States Department of Agriculture, Hilo, HI, United States, [5]Hawaii State Department of Health, Honolulu, HI, United States*

*Angiostrongylus cantonensis* is the most common cause of human eosinophilic meningitis. The infectious larva of this nematode develops in mollusks, but can also be present in paratenic hosts. The major route of infection in the US is thought to be unintentional consumption of infected mollusks in fresh produce or other foods. The aim of this study was to determine the prevalence of *A. cantonensis* larvae in mollusks and other hosts from Hawaii and continental regions of the US using quantitative real-time PCR. Slugs, snails and flatworms were collected from the environment and kept frozen until DNA extraction and real-time PCR analysis were performed. In order to estimate the parasitic burden in the samples studied, a standard curve was created using DNA extracted from preparations containing known numbers of *A. cantonensis* larvae. *Parmarion martensi* and other slugs and snails collected from an area of Hawaii associated with human cases of eosinophilic meningitis had a high percentage of samples with Ct values correlating to approximately 1000 larvae in 25 mg tissue. In contrast, channeled apple snails from five regions on continental US were virtually free of *A. cantonensis*, with the exception of a few snails collected around New Orleans that had Ct values correlating to less than 100 larvae in 25 mg tissue. In addition to these findings, *A. cantonensis* DNA was also detected in two species of Hawaiian flatworms, one of them tentatively identified as belonging to *Platydemus*. Further studies are needed to determine whether or not these flatworms may serve as vehicles for human angiostrongyliasis. In conclusion, this methodology can be successfully used in surveys of environmental samples to determine the geographical expansion of this parasite.

## 314

### EPIDEMIOLOGY OF HOOKWORM INFECTION IN KINTAMPO NORTH DISTRICT, CENTRAL GHANA

**Debbie Humphries**[1], Emily Mosites[1], Joseph Otchere[2], Amoani Twum[2], Lauren Woo[1], Hinckley Jones-Sanpei[3], Lisa Harrison[1], Richard D. Bungiro[4], Michael Wilson[2], Kwabena Bosompem[2], Michael Cappello[1]

*[1]Yale University, New Haven, CT, United States, [2]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [3]University of North Carolina, Chapel Hill, NC, United States, [4]Brown University, Providence, RI, United States*

A cross-sectional study evaluating the prevalence and intensity of soil transmitted nematode (STN) infections was carried out in 292 children and adults from 62 households in the Kintampo North District of the Brong Ahafo Region in central Ghana. Households were randomly selected from three communities. Based on Kato-Katz fecal microscopy, the overall prevalence of hookworm infection was 44.9%, with most infected individuals (93.0%) having light infections (<2000 eggs per gram). The prevalence of hookworm infection was lowest in children ages 5 and under (19.6%), peaked in ages 11-20 (59.5%), and decreased to 37.5% in those over age 60. The prevalence of other STNs (*Ascaris* and *Trichuris*) was less than 3%, while the prevalence of malaria infection was 54.4%. Risk factors for baseline hookworm infection in adults (age >15 yrs) included latrine access (p<0.01), shoe use (p<0.01), and being a farmer (p<0.01), when controlling for gender, age and community. Nutritional status was also significantly associated with baseline infection status, as 12.5% of adults with a BMI>23 were hookworm infected, compared to 56.2% of those with a BMI $\leq$ 23 (p<0.001). Factors associated with hookworm infection in children, controlling for gender, age and community, included co-infection with malaria (p<0.05) and increased serum reactivity to adult hookworm secretory antigens (p<0.05). Hookworm-infected subjects (n=104) were treated with a single dose of 400-mg albendazole and 97 (93%) were re-examined at 14-20 days post-treatment. Based on a follow-up fecal examination, albendazole treatment was associated with a cure rate of 62% and an overall egg reduction rate of 82%. However, among the 37 subjects (38%) whose follow-up sample remained positive for hookworm, there was no statistically significant reduction in fecal egg counts, raising the possibility of albendazole resistance. These data suggest a need for community based control programs to monitor efficacy and establish mechanisms to identify anthelminthic resistance in order to successfully reduce hookworm infection morbidity. The influence of nutritional status on susceptibility to hookworm infection also warrants further investigation.

## 315

### RELATIVE EFFECTIVENESS OF TWO ANTIHELMINTHIC REGIMENS TO CONTROL SOIL-TRANSMITTED HELMINTH INFECTIONS AMONG PRE-SCHOOL AGED CHILDREN IN BANGLADESH

**Cynthia Snider**[1], Masud Alam[2], Dinesh Mondal[2], William A. Petri, Jr.[1], Rashidul Haque[2]

*[1]University of Virginia, Charlottesville, VA, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh*

Chronic soil transmitted helminth infections (STHI) are associated with malnutrition, anemia, and impaired growth of children. Further research is needed to determine the most effective treatment regimen for STHI in high prevalence urban areas and measure its impact upon nutrition. This study was undertaken to compare the relative effectiveness of albendazole 400mg every 3 months versus conventional treatment of albendazole 400mg every 6 month to reduce prevalence, intensity of STHI and improve nutritional status in pre-school aged children in Bangladesh. Children aged 2-5 years old were randomly assigned to receive albdendazole 400mg at 3-month intervals versus 6-month intervals for a period of 12-months. The prevalence and intensity of infection as measured by mean egg burden in Ascariasis and Trichuriasis was the primary outcome. Secondary outcomes included height-for-age Z-score (HAZ), weight-for-age Z-score (WAZ), change in hemoglobin and episodes of diarrhea. A total of 194 children were observed for 12 months. The mean age was 41.8 months (SD, 1.11), and 47.4% were male. The 3-month regimen showed significant reduction in Ascaris prevalence from 56% to 24%.($p<0.0001$), as well as decrease in mean egg burden from 8136 +/- 1775 eggs per gram stool (epg) to 915 +/- 525 egp in comparison to conventional treatment ($P<0.0001$). No difference found between the 3-month and 6-month groups in the effectiveness of treating Trichuris, which was reduced from 79% in all children at baseline to 38.5% and 42.9% at one year, respectively ($p=0.54$). The only trend for nutritional improvement was seen in interventional group with a baseline WAZ of -1.92 which decreased to -1.88 at end of 12 months. No difference was seen in mean hemoglobin, and frequency of diarrhea between 2 groups at end of study ($p=0.8$). In conclusion, albendazole treatment in 3-month intervals reduced the prevalence and intensity of infection of Ascariasis more than the conventional regimen and showed trends of improved nutritional status in poor pre-school aged children.

## 316

### PERINUCLEAR PROTEIN, *P. FALCIPARUM* GAMETOCYTOGENESIS INDUCER 1, PFGYI1, PLAYS AN IMPORTANT ROLE IN GAMETOCYTOGENESIS

Saliha Eksi[1], Yoseph Haile[1], **Belinda J. Morahan**[1], Tetsuya Furuya[2], Amreena Suri[1], Hongying Jiang[2], Xinzhuan Su[2], Kim C. Williamson[1]

*[1]Loyola University Chicago, Chicago, IL, United States, [2]National Institutes of Health, Bethesda, MD, United States*

Although critical for malaria transmission, the molecular mechanisms regulating gametocyte production remain unknown. Comparative microarray analysis of genomic DNA isolated from gametocyte-producing (3D7.G+) and deficient (3D7.$G_{def}$) clonal *P. falciparum* lines identified an 18.93 kb deletion on chromosome 9 that contained a single open reading frame designated *P. falciparum* gametocytogenesis inducer 1 (*Pfgyi*1). Complementation of the 3D7.$G_{def}$ line with *Pfgyi1* restored gametocytogenesis demonstrating its importance in gametocyte production. Additionally, 32 genes were found to be differentially up regulated during early gametocytogenesis, *P. falciparum* gametocyte early (*Pfgye*) genes, in the 3D7.G+ clone compared with the 3D7.$G_{def}$ line. Complementation of 3D7.$G_{def}$ with *Pfgyi* restored expression of the *Pfgye* genes tested confirming their dependence on *Pfgyi*. Neither *Pfgyi1* nor the *Pfgye* genes have close orthologs in yeast or metazoans, suggesting that *Plasmodium spp.* utilize a unique differentiation pathway. Green fluorescent protein (GFP)-tagged PfGYI1 revealed a perinuclear expression pattern similar to that observed for PfSir2, which is involved in regulating subtelomeric gene expression. However, GFP-tagged PfGYI1 is only expressed in the subpopulation of trophozoites and schizonts that are committed to gametocytogenesis. The perinuclear location of PfGYI1 together with homology to transport regulators suggests that PfGYI1 may induce gametocytogenesis by playing a role in the nuclear targeting of gametocyte-specific regulators.

## 317

### A METHOD FOR *IN VITRO* PRODUCTION OF *P. FALCIPARUM* OOKINETES REVEALS NOVEL INSIGHTS INTO OOKINETE BIOLOGY

**Viengngeun Bounkeua**, Fengwu Li, Joseph M. Vinetz

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

Malaria is a blood-borne disease that is caused by the Plasmodium parasite and transmitted by female Anopheline mosquitoes. Despite advances in pharmacology and preventive health, malaria remains a leading cause of morbidity and mortality in the developing world. Transmission of malaria from vertebrates to mosquitoes depends on the successful development in and invasion of the mosquito midgut by the Plasmodium ookinete. Blocking this process, via transmission-blocking interventions, can limit or stop the cycle of malaria re-infections in endemic regions. Comprehensive understanding of ookinetes from the lethal human parasite, *Plasmodium falciparum*, has, until this point, been inferred from findings in ookinetes of animal-infecting Plasmodium ssp. Direct study of human Plasmodium ookinetes has been hampered by the inability to produce sufficient quantities *in vitro*. We report an efficient, *in vitro* method of producing and purifying *P. falciparum* mosquito-stage forms from blood-stage gametocytes. We typically generate *P. falciparum* gametocyte cultures of 6-8% total gametocytemia (5-6% stage V), and yields of 5-20 x 107 gametocytes per 25mL culture volume, which is a 2-5 fold increase over typical gametocytemias of 1-2%. Our ookinete cultures have a typical gametocyte-to-ookinete transformation rate of 25% with rates of up to 42% observed; yields range from 5-60 x 106 ookinetes per 10mL culture volume. Ookinetes were identified by Giemsa-stained light microscopy, immunofluroescence microscopy and transmission electron microscopy. The transmission electron micrographs of these cultured *P. falciparum* parasites irrefutably demonstrate evidence that they are ookinetes and reveal novel insights into *P. falciparum* ookinete, the to be demonstrated for the human malaria parasite. The methods described are a major breakthrough for the field and are applicable for the development of transmission-blocking vaccines, which prevent infection of the mosquito vector by the vertebrate host, and large-scale *in vitro* production of sporozoites, which is currently not feasible.

## 318

### CELLULAR AND MOLECULAR INTERACTIONS WITH HEPARIN-LIKE MOLECULES DURING INVASION OF ERYTHROCYTES BY *PLASMODIUM FALCIPARUM* MEROZOITES

Michelle Boyle[1], Jack S. Richards[1], Paul R. Gilson[2], Wengang Chai[3], **James G. Beeson**[1]

*[1]Walter and Eliza Hall Institute of Medical Research, Parkville, Australia, [2]Macfarlane Burnet Institute for Medical Research and Public Health, Melbourne, Australia, [3]Glycosciences Laboratory, Imperial College London, Northwick Park and St. Mark's Campus, Harrow, United Kingdom*

Understanding the molecular and cellular mechanisms of *Plasmodium falciparum* merozoite invasion is an important aim in the search for vaccine candidates and novel antimalarials. Heparin and related sulfated polysaccharides are known to inhibit *P. falciparum* blood-stage growth. However, their mechanism of action and the structural requirements of heparin-like molecules for inhibitory activity are not known. By studying merozoite invasion with live video microscopy, we have shown that heparin acts by blocking initial contact between the merozoite and

erythrocyte, but has little effect on other stages of the life-cycle. Heparin and related molecules inhibited all isolates tested and there was no difference in activity when parasites used different invasion phenotypes. Using polysaccharides with defined sulfate content and pattern, or with different forms of uronic acid, we have defined the key structural features of heparin-like molecules required for inhibitory activity and determined the minimum chain length for activity. Furthermore, we have identified a merozoite protein that specifically binds heparin, which appears to be the target of heparin's invasion-inhibitory activity. Heparin-like molecules occur naturally on the surface of human erythrocytes, and may function as receptors for binding of merozoite surface antigens during invasion. These findings have significant implications for understanding *P. falciparum* invasion of erythrocytes, identifying antigens that could developed as vaccine targets, and for developing novel antimalarials.

## 319

### THE INFLUENCE OF PAIRING ON GENE EXPRESSION OF *SCHISTOSOMA MANSONI* ADULT FEMALES

**Giulliana T. Almeida**[1], Thiago M. Venancio[1], Ricardo DeMarco[2], Sergio Verjovski-Almeida[1]

*[1]Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, Sao Paulo, Brazil, [2]Departamento de Física e Informática, Instituto de Fisica de São Carlos, Universidade de São Paulo, São Carlos, Brazil*

*Schistosoma mansoni* is one of the causative agents of schistosomiasis, a major health problem in developing countries. The parasite survives in the human bloodstream and male-female pairing is an essential pre-requisite for the completion of female growth and reproductive morphogenesis. Classical studies show that the direct contact with male is required to achieve and maintain female maturity. To investigate and understand more about the influence of male-female interaction on gene expression, adult schistosomes from mixed infections were recovered by perfusion and two groups of adult female worms were maintained at *in vitro* culture. One group was formed by females separated from males; the other by worm pairs. After 24 hours the female worms (single and paired) were collected and total RNA extracted. To identify differentially expressed genes we performed large-scale gene expression analysis using in-house constructed 4,000-element cDNA microarrays. Gene expression analysis of females from worm pairs relative to female worms kept in culture without male revealed a significant (FDR = 5%) down-regulation of 75 genes and up-regulation of 35 genes. Among down-regulated genes, many of them encode surface antigens and proteins associated with immune signals, suggesting an important and specific strategy evolved in *Schistosoma* to reduce the actual damage from the host's immune system, besides an adaptation of the female to survive in the absence of male.

## 320

### UNDERSTANDING THE BIOLOGY OF SCHISTOSOMES IN RESPONSE TO PRAZIQUANTEL

Pauline M. Cupit[1], Anthony D. Aragon[1], Tinopiwa Goronga[2], Thomas R. Webb[2], **Charles Cunningham**[1]

*[1]University of New Mexico, Albuquerque, NM, United States, [2]St. Jude Children's Research Hospital, Memphis, TN, United States*

While a number of theories regarding the binding partner and mode of action of praziquantel (PZQ) have been advanced since its discovery over 30 years ago, none have been proven definitively. PZQ is the only widespread means of schistosome treatment and control used currently and there is concern that resistance may become a serious problem. Understanding how the drug works has the potential to provide insights into how resistant schistosome isolates evolve and how a new generation of therapeutics might be developed. Our objective is to identify the binding partner and molecular pathway(s) on which PZQ acts. Glutathione, adenosine receptor, actin and myosin light chain, among others have each been suggested as the binding target of PZQ. None of these, however, easily explain the greatly reduced PZQ sensitivity of juvenile schistosomes or schistosomes grown a single sex infection. We have developed an innovative method to identify the binding partner of PZQ through 'activity based profiling' using a PZQ probe containing a diazarine group to covalently cross link the drug to its target and an alkyne group to which a reporter tag can be attached using click chemistry. Probe bound target is then identified by chemiluminescent detection. Using this technique we have identified a number of protein bands that may contain the PZQ target. We are currently characterizing these molecules through protein sequencing and expression analysis. We have previously conducted a transcriptomal analysis of gene expression between PZQ insensitive juvenile and PZQ sensitive mature *S. mansoni* that revealed 607 up-regulated candidate genes in mature schistosomes whose products are potential PZQ targets. A comparison of this gene list with that of genes expressed by PZQ sensitive miracidia reduced this target list to 247 genes. These genes included a number involved in aerobic metabolism and cytosolic calcium regulation. We are now extending this microarray work to include other PZQ sensitive life cycle stages in the hope of reducing the number of genes common to PZQ sensitive stages even further and identifying the metabolic pathway(s) targeted by the drug. Using these innovative approaches we believe our work will aid clarification of the impact of PZQ on schistosomes and help ensure we continue to have effective means for treatment and control of this neglected tropical disease.

## 321

### INVESTIGATION OF AN OUTBREAK OF SUPPOSED LEPTOSPIROSIS IN PEDRO CANÁRIO, ESPÍRITO SANTO STATE, BRAZIL, JANUARY, 2009

**Verena M. Souza**[1], Juliano L. Hoffmann[1], Maxwell Marchito[2], Wildo N. Araújo[1]

*[1]Ministry of Health, Brasilia, Brazil, [2]Secretariat of Health of Espírito Santo State, Vitória, Brazil*

Between 2000-2008, no cases of leptospirosis were notified from town of Pedro Canário, Espírito Santo, Brazil. In November 2008 the town intensified leptospirosis surveillance, instructing every suspect case of dengue fever be tested for leptospirosis. Consequently, by 01/23/2009, 169 suspect leptsospirosis cases were reported, with fever, headache and myalgia; 17 were confirmed for leptospirosis by ELISA-IgM. We investigated this apparent outbreak of leptospirosis. The objective of this sttudy was to confirm the outbreak, define the magnitude of the event and describe it by person, time and place. For suspected cases, we reviewed investigation forms and laboratory data, conducted home visits to collect repeat clinical samples and document signs and symptoms using a standardized questionnaire and conducted home-inspections. Of 169 suspect cases, 123 tested positive for dengue fever and were ruled out as leptospirosis infections; 46 suspect cases had inconclusive laboratory tests. Of these 29 (63%) had fever and headache, 32 (70%) myalgia, 17 (37%) intense purities and rash, 16 (35%) vomit and 9 (20%) retro-orbital pain. None had been treated with antimicrobials and there were no hospitalizations or deaths. In 60% the yards of homes visited, we observed trash, 40% bottles, 20% tires, 35% cans, 50% garbage and 65% buckets and plant pots. In some cases, all family members were ill simultaneously. The health department reported *Aedes aegypti* domicile infestation rates of 1.3% to 10.6%. The National Dengue Surveillance System showed an increase in cases between 2001-2008. In conclusion, an outbreak of dengue fever occurred in the city; false-positive leptospirosis diagnostic test results precipitated the report a non-existent outbreak. Among febrile illnesses not confirmed as dengue fever, a confluence of evidence suggests dengue fever, not leptospirosis, as the cause. We recommend that in periods of dengue outbreaks, where no precedents indicated the need for increased diagnosis, that enhanced laboratory testing for leptospirosis not be undertaken.

## 322

### HYPOGLYCEMIA IS ASSOCIATED WITH MORTALITY IN UGANDAN PATIENTS WITH SEVERE SEPSIS

**Richard Ssekitoleko**[1], Christopher C. Moore[2], Shevin T. Jacob[3], Relana Pinkerton[2], Patrick Banura[4], David Meya[5], Steven J. Reynolds[6], Nathan Kenya-Mugisha[7], Harriet Mayanja-Kizza[5], W. Michael Scheld[2]

*[1]Mbarara University, Mbarara, Uganda, [2]University of Virginia, Charlottesville, VA, United States, [3]University of Washington, Seattle, WA, United States, [4]Masaka Regional Referral Hospital, Masaka, Uganda, [5]Makerere University, Kampala, Uganda, [6]National Institutes of Health, Bethesda, MD, United States, [7]Ministry of Health, Kampala, Uganda*

Sepsis is an increasingly recognized cause of death in sub-Saharan Africa. Because dysglycemia is associated with poor outcomes in patients with sepsis, point of care glucose measurement may improve assessment and treatment of patients with sepsis in resource limited settings. We enrolled 382 patients in a prospective observational study of severe sepsis in Uganda. Inclusion criteria were age ≥18 years and admission to a medical ward, along with 1) ≥ 2 of the following: body temperature > 38°C or < 36°C; heart rate > 90 beats/minute; or respiratory rate >20 breaths/minute, or thermodysregulation alone; 2) a systolic blood pressure (SBP) ≤ 100 mmHg, and 3) a suspected infection. Exclusion criteria included acute cerebrovascular events, gastrointestinal hemorrhage, or admission to a non-medical ward. Whole blood glucose was measured at the time of enrollment. The results of this study have shown that: 1) Of 382 patients enrolled in our study, we had available glucose data for 323; 2) 101 of 322 (26.4%) had dysglycemia with 32 of 322 (8.4%) having hypoglycemia (<70 mg/dL) and 69 of 323 (18.1%) having hyperglycemia (>150 mg/dL); 3) There was a statistically significant relationship between dysglycemia and in-hospital death with 30 of 101 (29.7%) patients with dysglycemia dying in hospital versus 43 of 222 (19.5%) of patients without dysglycemia dying in-hospital (Odds Ratio 1.749; 95% Confidence Interval 1.018-3.006; p = 0.042); 4) There was a statistically significant relationship between hypoglycemia and in-hospital death with 13 of 32 (40.6%) patients with hypoglycemia dying in-hospital versus 60 of 290 (20.7%) of patients without hypoglycemia dying in hospital (Odds Ratio 2.623; 95% Confidence Interval 1.226-5.611; p = 0.011) and 5) There was not a statistically significant relationship between hyperglycemia and in-hospital death with 17 of 69 (24.6%) patients with hyperglycemia dying in-hospital versus 56 of 253 (22.1%) of patients without hyperglycemia dying in-hospital (Odds Ratio 1.15; 95% Confidence Interval 0.617-2.144; p = 0.662). In conclusion, dysglycemia, particularly hypoglycemia, is associated with poor outcomes in patients presenting with severe sepsis in Uganda. Glucose monitoring may be a cost effective method of triaging critically ill patients and maintenance of euglycemia may improve outcomes.

## 323

### CLINICAL OUTCOMES IN HOUSEHOLD CONTACTS OF PATIENTS WITH CHOLERA IN BANGLADESH

**Ana A. Weil**[1], Ashraful I. Khan[1], Fahima Chowdhury[1], Regina C. LaRocque[2], A. S. Faruque[1], Edward T. Ryan[2], Stephen B. Calderwood[2], Firdausi Qadri[1], Jason B. Harris[2]

*[1]International Center for Diarrheal Disease and Research, Bangladesh, Dhaka, Bangladesh, [2]Massachusetts General Hospital, Boston, MA, United States*

Multiple *Vibrio cholerae* infections within the same household are common. In this study we examine the temporal relationship between infection of index cases and household contacts, and identify risk factors for development of severe disease in household contacts of patients with severe cholera. Household contacts of hospitalized patients with cholera were observed with frequent clinical assessments and collection of serum and rectal swab cultures for a period of 21 days after presentation of the index case. Half (460/944) of all contacts reported diarrhea during the study period, and symptoms most frequently began two days after presentation of the index case. Antibiotics were used by 43% (199/460) of the contacts with diarrhea. Rectal swab culture for *V. cholerae* was positive in 22% (202/944) of contacts and 73% (148) of infected contacts experienced diarrhea. Moderate to severe dehydration developed in 26 contacts; predictors of dehydration included vomiting, each additional day of diarrhea, and blood group O status. In urban Bangladesh, the burden of diarrheal illness in household contacts of cholera patients is higher than previously estimated and prophylactic intervention is feasible because the majority of symptomatic cases of *V. cholerae* infection in contacts began after presentation of the index case. Reconsideration of targeted chemoprophylaxis for household contacts of cholera patients may be warranted.

## 324

### OUTBREAK OF MASS SOCIOGENIC ILLNESS IN SECONDARY SCHOOLS IN BANGLADESH ASSOCIATED WITH MEDIA COVERAGE AND PERSON-TO-PERSON TRANSMISSION

**Emily S. Gurley**[1], Mahmudur Rahman[2], Nazmun Nahar[1], M. Jahangir Hossain[1], Rebeca Sultana[1], Nadia Ali Rimi[1], M. Saiful Islam[1], Main Uddin[1], Dawlat Khan[1], Mushtuq Husain[2], Musleh Uddin[2], Shamsi A. Chowdhury[2], Nuzhat N. Banu[2], Stephen P. Luby[1]

*[1]International Center for Diarrhoeal Disease Research, B, Dhaka, Bangladesh, [2]Institute for Epidemiology, Disease Control and Research, Dhaka, Bangladesh*

During the summer of 2007, 20 secondary schools from 16 districts in Bangladesh reported outbreaks of illness in students which included symptoms of dizziness, nausea, recurrent unconsciousness and abnormal movement of limbs. We investigated with the objectives of determining the etiology of and exposures associated with disease. Patients treated by physicians underwent physical examination and their blood specimens were analyzed for signs of disease. The index school was examined for potential toxic contamination. We conducted in-depth interviews and group discussions with case-patients, their guardians and teachers from the index school to generate hypotheses about exposures associated with illness and then used a cross-sectional survey at 4 affected schools to investigate associations between illness and exposures. Physicians and psychiatrists examined approximately 30 hospitalized patients from the index school and found no physical abnormalities; blood samples collected from 17 patients showed normal total and differential count of white blood cells serum electrolytes, liver function and kidney function. The investigation team observed no evidence of environmental contamination. Individual and community perceptions about the cause of the outbreak included inclement weather, bioterrorist attack, revenge from supernatural forces, stressful relationships between school staff and students, and personal stress. Nineteen additional schools reported outbreaks after widespread media coverage of events at the index school. Patients commonly reported having headache (74%), weakness or drowsiness (66%), vertigo (65%), and crying (64%). Illness was associated with high stress levels, smelling a strange odor, and seeing and caring for others with this illness; females were 2.5 times more likely than males to become ill. In conclusion, the absence of clinical findings in patients and the wide range of presenting symptoms, coupled with "line of sight" transmission, suggests that this was an outbreak of mass sociogenic illness. This outbreak caused widespread panic in affected communities and large teams worked for weeks to respond to this outbreak, taking away from other disease surveillance activities. Experience from this outbreak shows that sensationalism of outbreaks in the media can have negative health consequences for communities.

## 325

### SPATIAL PATTERNS OF MENINGITIS IN NIGER

**Nita Bharti**[1], Helene Broutin[2], Rebecca Grais[3], Ali Djibo[4], Bryan Grenfell[1]

*[1]Penn State University, University Park, PA, United States, [2]Fogarty International Center, National Institutes of Health, Bethesda, MD, United States, [3]Epicentre, Paris, France, [4]Direction Generale de la Sante Publique, Ministere de la Sante, Niamey, Niger*

In Africa, meningitis outbreaks occur only during the dry season. Previous analyses from Niger have suggested that population density peaks during the dry season and that this is strongly correlated with increased transmission of measles. We propose that the strong seasonality in meningitis incidence is similarly affected by seasonal fluctuations in host aggregation. Although climatic factors are widely believed to play a role in meningitis seasonality, here we specifically focus on the potential role of human movement and density. A strong environmental component to meningitis dynamics would lead us to predict a correlation in meningitis within rainfall contours. However our analysis shows that spatial patterns of meningitis fadeouts and reintroductions are more highly clustered in regions along primary roads and migration corridors, suggesting that population density also contributes to the spatiotemporal spread of meningitis. We further show that districts in Niger with high meningitis reintroduction rates also have high measles reintroduction rates. In spite of the epidemiological differences between the two diseases, human movement patterns can be seen in the spatial dynamics of both. This analysis gives us a better understanding of regional contact patterns and disease dynamics to identify areas important for disease surveillance and vaccination.

## 326

### RE-EMERGENCE OF CHIKUNGUNYA FEVER IN NARATHIWAT PROVINCE : A STUDY ON CLINICAL MANIFESTATIONS

**Thongchai Lertwilairatanapong**, Jariya Narathipput, Suvimon Heng

*Narathiwat Provincial Health Office, Narathiwat, Thailand*

Since chikungunya virus (CHIKV) infection was first reported in Bangkok in 1958,and disappeared in the late 1970s, there have been small localized outbreaks in 7 isolated provinces between 1976 and 1995. In September 2008, Narathiwat Provincial Health Office was notified by Community hospitals of suspected chikungunya fever.This re-emergence of CHIKV has spread to other 4 adjacent provinces. Chikungunya fever cases which were admitted in hospitals from September to November 2008 were investigated. Epidemiological and clinical data were collected from 64 confirmed CHIKV infection and were analyzed by Epi Info. CHIKV was isolated from both *Aedes albopictus* and *Ae. aegypti* mosquitoes. Majority of the cases were adult s(87%) with the mean age of 41.1 years. The male to female ratio was 1:1.4. Most of them were wage earners (37%), rubber plantation workers and farmers (26.6%). Major clinical manifestations include fever (84.4%), arthralgia (82.8%), headache (54.7%), rash(40.6%). Onset is usually abrupt with high fever, 50% of cases had the temperature higher than 39°c.The duration of fever ranged from 1 to 4 days (mean 2.9 days). Arthralgia usually involved multiple small joints and were migratory. Joint swelling was noted in only 3% of cases. Skin rashes are erythrematous and maulopapular with itching (80.8%).The CBC were normal in most cases.The treatment was entirely symptomatic. Acetaminophen was used in 95.3% for fever and pain while NSAID was used in 64% for severe arthralgia.The length of stays in hospital ranged from 1 to 5 days(mean 2.6 days). In conclusion, this outbreak has spread to adjacent provinces wider than before. The clinical manifestations are similar to other previous reports. There were however some differences which include lower incidence of joint swelling and higher incidence of skin itching. The clinical triad of acute onset of fever, arthralgia and rash are useful for surveillance of CHIKV infection. The presentation of multiple and small joint pain with joint swelling help in differential diagnosis from dengue fever.

## 327

### DIABETIC RETINOPATHY IN AN URBAN DIABETIC CLINIC IN MALAWI

**Simon J. Glover**, Theresa J. Allain, Danielle B. Cohen

*College of Medicine, Blantyre, Malawi*

Diabetes is increasing in prevalence in resource poor countries where it is under diagnosed and under treated. Present healthcare systems struggle to cope with this chronic serious disease. Diabetic retinopathy is a microvascular complication of diabetes that can severely affect the vision of diabetics of all ages, often during the peak years of their professional lives. Early diagnosis and treatment of diabetic retinopathy improves visual outcome. The purpose of this study was to record the prevalence and severity of retinopathy in a diabetic population in an urban diabetic clinic in Malawi. We recruited 279 consecutive new patients who had not undergone systematic retinal assessment before from the diabetic clinic in Queen Elizabeth Central Hospital in Blantyre, Malawi. All patients were examined by 1 experienced ophthalmologist who graded retinopathy using a slit lamp after pupil dilation. 26.9% had at least mild pre proliferative retinopathy or any maculopathy or both. 21.1% had sight threatening eye disease (STED). 12.9% had STED affecting the macula. 11.8% had STED affecting the retina. 2.5% had active proliferative retinopathy. 3.6% (10/279) had fibrovascular proliferation, of which 5 had tractional retinal detachment, 3 with active proliferation. 9% had background diabetic retinopathy only. 64.1% had no diabetic retinopathy. In conlcusion, we found a significant level of treatable diabetic retinopathy in a previously unscreened population. Many patients would have benefitted from laser treatment, which is not available in Malawi.

## 328

### CORRELATION OF DERMATOLOGICAL AND OPHTHALMOLOGICAL MORBIDITY IN ONCHOCERCIASIS (FOREST TYPE)

**Luc E. Coffeng**[1], Wilma A. Stolk[1], Sake J. de Vlas[1], Michel Boussinesq[2], Grace N.A. Fobi[2], Gladys A. Ozoh[2], Peter A. Enyong[2], Anne-Cécile Z.-K. Bissek[2], Dik J.D.F. Habbema[1]

*[1]Erasmus Medical Center, Rotterdam, The Netherlands, [2]African Programme for Onchocerciasis Control, Ouagadougou, Burkina Faso*

Although there is ample information about the prevalence of skin and eye disorders in African onchocerciasis (river blindness), it is unknown to what extent these two disease entities occur simultaneously in individual patients. Apart from a perspective of pure biological interest, concomitance of dermatological and ophthalmological morbidity is also important when estimating the global burden of disease (GBD) of onchocerciasis. The current study investigated the possible occurrence of specific combinations of morbidity in onchocerciasis (forest type). Dermatological and ophthalmological data from a cross-sectional, population-based survey (Cameroun, 1998) were matched at the individual level (N = 840). Only individuals who had been in the area for 3 months or longer were included in the analysis (N = 765). At the time of survey, the source population was still naive for mass treatment with ivermectin. However, 30% of the population reported having used ivermectin at some point in time. Data was analysed using logistic regression, while adjusting for study site, age, gender, social economical status (SES), ivermectin use and several proxies for exposure to *Onchocerca volvulus*. Onchocerciasis was highly endemic (nodule prevalence 65% in men aged 20 years and above). The prevalence of visual impairment (low vision or blindness), troublesome itch and skin depigmentation were 9%, 17% and 23% respectively. All three were strongly associated with the presence of nodules. Troublesome itch was not associated with blindness (odds ratio 0.7; 95%-CI: 0.3 - 1.9) or depigmentation (0.8; 0.5 - 1.5). However, there was a significant association between skin depigmentation

and visual impairment (any), even after adjusting for study site, age, gender, SES, ivermectin use and the presence of nodules, microfilariae in the anterior chamber of the eye, reactive skin lesions and troublesome itch (2.2; 1.2 - 4.3). In conclusion, there seems to be a predisposition towards the co-occurrence of skin depigmentation and visual impairment (any) in onchocerciasis of the forest type. When including the impact of skin depigmentation in future estimations of the GBD of onchocerciasis, an overestimation of the GBD should be avoided by taking into account the overlap with visual impairment.

## 329

### PATIENT TREATMENT COSTS FOR MANAGEMENT OF LYMPHEDEMA AND ACUTE ATTACKS IN TOGO

**Paul T. Cantey**[1], Stephanie Richard[2], Stephanie Richard[3], Ameyo Dorkenoo[4], Yao Sodahlon[5], Els Mathieu[1]

*[1]Division of Parasitic Diseases, NCZVED, Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Fogarty International Center, Bethesda, MD, United States, [3]John Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [4]Togo National Program for the Elimination of Lymphatic Filariasis, Lome, Togo, [5]Mectizan Donation Program, Decatur, GA, United States*

Lymphatic filariasis (LF) is a major contributor to disability in the developing world. Togo is a West African country with 1.1 million persons living at risk for LF and high prevalence of poverty, with 61.7% of the population living on less than US$1.30 per day. This cohort survey was designed to collect baseline data in 2005, before the implementation of a national lymphedema program, and had to be redone in 2007 due to a delay in release of program funding. A convenience sample of 188 lymphedema patients from six LF endemic districts was enrolled. The survey questions covered many LF-related issues, but this abstract focuses on questions related to the cost of treatment of lymphedema and acute attacks, and family wealth. Questions were translated from French into the local language by interviewers who entered the responses into personal digital assistants. Cost data were recorded in the local currency and, for in-kind payments, animal values were estimated based on market costs. The total cost estimates include payments to the provider, as well as cost of treatments and treatment related travel. The 2005 data are presented here; 2007 data will be included in the final presentation. A total of 188 patients with leg lymphedema were included in the analysis, and costs for lymphedema management and treatment of acute attacks were estimated separately. Cash payments to medical providers comprised 44% of the cost of lymphedema management prior to implementation of a lymphedema program. Only traditional healers accepted animals for payment (27; 20.3%). The median annual total cost for treatment of lymphedema was US$5.00 (range US$0.00-US$1,173.07). Payments for materials comprised 75% of the cost of acute attacks. Animals were accepted as payment for several traditional therapies but not for pharmaceuticals. The median total cost for treatment of acute attacks was US$1.92 (range US$0.00-US$336.54). Most patients (124; 72.5%) reported a change in occupation due to their lymphedema, with 59 (34.5%) working less time and 48 (28.1%) stopping work altogether. This is the first report detailing lymphedema treatment costs in Africa. Given the extreme poverty of Togo and much of sub-Saharan Africa, alleviating the chronic manifestations of LF could contribute significantly to the economic well-being of those people living in endemic areas. Data to determine the impact of lymphedema management programs on the cost to patients of chronic LF are needed.

## 330

### THE WEST AFRICAN LF MORBIDITY PROJECT: TRAINING IN LF SURGERY AS AN AVENUE TO LYMPHATIC FILARIASIS ELIMINATION

**Anders R. Seim**[1], Sunny D. Mante[2], Serigne M. Gueye[3]

*[1]HDI (Health and Development International), Fjellstrand, Norway, [2]Ghana Army Medical Corps, Accra, Ghana, [3]University Cheikh Anta Diop, Dakar, Senegal*

To disseminate WHO-recommended LF-hydrocele (filaricele) surgery where postgraduate medical education is inadequate, The West African LF Morbidity Project was started in 2004. 25 million LF patients have scrotal fluid, often enough to be socio-economically incapacitating. In some areas, 25%-50% of men have filaricele. One and now 2 urologists do workshops for teachers of surgery, district surgeons, etc. Through 2008, 323 were trained in 12 African countries, and at least 3118 patients treated. In 2006, an external evaluation was done in 3 countries. For Ghana, a socio-economic survey of ex-patients was made by the Atlanta LF-Support Center and CDC. In Burkina Faso and Togo, head of surgery at the Dakar medical school who later joined the project (S.M.G.) interviewed surgeons, led focus-groups, and interviewed and clinically examined convenience-sampled patients. Conclusions of the evaluation and observations in 2 additional countries have suggested: (1) LF-hydrocele (filaricele) repair as recommended by WHO is popular among surgeons and patients, easy to learn, easy to do, requires shorter hospital stays, and gives excellent clinical and socio-economic results when done well in West African district hospital settings. Surgeons express surprise and pleasure at how much better the new procedure is than what they used previously; (2) When antibiotic cover is not properly implemented, patients living far away leave before skin seals, or patients do not receive adequate care until skin integrity is reestablished in about 7 days, then unacceptable rates of postoperative infections, often mild but sometimes catastrophic, regularly occur in West African district hospital settings; (3) Attention to details of the procedure (e.g. careful skin closure without gaps, applying the recommended bandaging technique, and antibiotic cover pre- and post-operatively) makes a major difference to LF-surgery outcomes in African district hospitals; and (4) Surgical "campaigns" can have disadvantages and may lead to worse outcomes than the same surgeons achieve routinely in the same under-resourced settings.

## 331

### A RODENT MODEL OF LYMPHATIC PATHOLOGY DUE TO ADULT FILARIAL WORMS

**Charles D. Mackenzie**[1], Allison Eavey[1], Tiffany Weinkopff[2], Pat Lammie[2], Timothy Geary[3]

*[1]Michigan State University, East Lansing, MI, United States, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]McGill University, Montreal, QC, Canada*

The range of pathological and constitutional tissue changes that occur in lymphatic vessels associated with the presence and death of adult filarial worms remains relatively poorly understood. A rat model of infection with Brugia sp induces changes in the tissues that appear to be similar to those seen in humans with filarial parasites. In this study standard histochemical and special stains were used to identify specific components and changes in the progression of pathological changes in and around the lymphatics containing the adult parasites. The tissue changes involved extensive alterations in different components of the vessels; endothelial polyps, valvular proliferation, and cellular infiltrates, both peri-vascularly and within the wall tissues. B cell and eosinophil responses predominated the cellular infiltrates associated with the presence of living adult worms. Increases in the cells were associated with parasite stage change and the initiation of microfilariae release. The changes associated with dead or dying adult worms had a different immunocytochemical profile to that seen with the living parasites; a distinct profile of T cell subsets and monocytes were associated with degenerating adult worms.The similarity

with changes seen in human infections and the consistent nature of these changes indicate that this model can be used to provide information on the pathogenesis of lymphatic pathology due to filariasis. A comparison of these changes with those occurring in a range of different human filarial parasites will also be made.

## 332

### HIGH DOSE BIANNUAL ALBENDAZOLE AND IVERMECTIN SUPPRESS *WUCHERERIA BANCROFTI* MICROFILARIAL LEVELS MORE EFFECTIVELY THAN STANDARD DOSE ANNUAL TREATMENT

**Yaya I. Coulibaly**[1], Benoit Dembele[1], Siaka Konate[1], Housseini Dolo[1], Siaka Y. Coulibaly[1], Dramane Sanogo[1], Lamine Soumaoro[1], Michel E. Coulibaly[1], Salif S. Doumbia[1], Abdallah A. Diallo[1], Sekou F. Traore[1], Adama D. Keita[2], Thomas B. Nutman[3], Amy D. Klion[3]

*[1]University of Bamako, Bamako, Mali, [2]Hospital of Point G, Bamako, Mali, [3]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States*

Annual mass treatment with albendazole and ivermectin is the mainstay of current strategies to interrupt transmission of *Wuchereria bancrofti* (Wb) in Africa. More effective microfilarial suppression could potentially reduce the time necessary to interrupt transmission and ease the economic burden of mass treatment programs in countries with limited resources. To determine the effect of increased dose and frequency of albendazole/ivermectin (A/I) treatment on microfilarial (mf) clearance, 40 Wb microfilaremic residents of an endemic area in Mali were randomized to receive three doses of standard annual A/I therapy (400 mg/150 mcg/kg; n=21) or six doses of biannual increased dose A/I therapy (800 mg/400 mcg/kg; n=19). Mf levels were assessed by Nuclepore filtration of 1 ml of blood and circulating antigen (CAg) levels by TropBio™ ELISA. We have previously reported the results at 6 and 12 months, which demonstrated a significant decrease in mf counts in both groups with complete clearance of detectable mf at 12 months in all 19 subjects in the biannual group as compared to 10/21 in the annual group ($p<0.001$, Fisher's exact test). This difference between the two groups was sustained at 18 months with no detectable mf in the subjects receiving biannual treatment as compared to 6/21 in the annual treatment group ($p<0.001$). A significant and comparable decrease in CAg levels was seen in the annual and biannual treatment groups at 12 months with geometric mean (GM)% pre-treatment levels of 25% and 37%, respectively. Although GM levels rose in both groups at 18 months to 35% and 58% of pre-treatment levels, this difference was not statistically significant. There was no significant change in the number of worm nests detected in either group by ultrasound at 12 months as compared to baseline. These findings suggest that increasing the dose and frequency of treatment enhances the efficacy of A/I therapy at suppressing microfilarial levels, although longer therapy may be necessary to demonstrate a significant effect on adult worms.

## 333

### SCREENING OF APPROVED DRUGS FOR EFFICACY AGAINST THE *WOLBACHIA* ENDOSYMBIONT OF FILARIAL NEMATODES

**Kelly L. Johnston**[1], Indira Umareddy[2], Florence Feby Cahya[2], Ralf Altmeyer[2], Mark J. Taylor[1]

*[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]CombinatoRx Singapore, Singapore*

Lymphatic filariasis and onchocerciasis are debilitating diseases caused by parasitic filarial nematodes, which harbour an essential bacterial endosymbiont, *Wolbachia*. These bacteria are considered to be an important drug target in the chemotherapy of filariasis as their depletion affects both worm survival and fecundity. Doxycycline is an effective sterilising and macrofilaricidal agent but the duration of treatment required and contra-indications make it unsuitable for use in mass drug administration (MDA). The Anti-*Wolbachia* (A-WOL) Consortium aims to identify a novel anti-*Wolbachia* compound or combination that is suitable for use in MDA. A 96-well format cell-based assay for screening novel drugs active against *Wolbachia* has been developed utilising a *Wolbachia*-containing *Aedes albopictus* cell line (C6/36Wp) and quantitative PCR as the assay readout. In addition to performing low throughput screening of tetracycline derivatives and other compounds with predicted efficacy based on our target discovery objective, this assay has also been further optimised in order to examine the anti-*Wolbachia* effects of approved drugs singly and in combination. Using doxycycline as a gold-standard, critical features such as reproducibility and assay duration were evaluated as well as dynamic range and, having achieved acceptable Z' factors, the assay was validated for use. Results from a single agent screen of a selection of the human pharmacopeia (~2600 drugs) have revealed that approved drugs from various classes show significant bacterial load reduction equal to or superior to the gold-standard doxycycline. A selection of these hits has entered the A-WOL primary *in vivo* nematode screen (*Litomosoides sigmodontis* in the mouse) and further screening of the *Wolbachia* cell-based assay using approved drugs in combination with doxycycline (a doxycycline-enhancer screen) is underway. This work indicates that re-purposing of approved drugs could offer a novel approach to identify future treatments against filarial diseases.

## 334

### EXPRESSION OF *PLASMODIUM FALCIPARUM* ERYTHROCYTE MEMBRANE PROTEIN 1 (PFEMP1) IS IRON-DEPENDENT

**May Ho**, Steven D. Beaudry, Aaron White, Kristine Lee, Takayuki Arie, Kirk Deitsch, Rick M. Fairhurst

*Department of Microbiology and Infectious Diseases, University of Calgary, Calgary, Alberta, Canada, Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States, Department of Microbiology and Immunology, Weill Medical College of Cornell University, New York, NY, United States*

The binding of *P. falciparum*-infected red cells (IRBC) to host cells is mediated by the cytoadherence ligand PfEMP1. The factors which regulate PfEMP1 expression have not yet been determined. The growth, multiplication and virulence of a number of microbial pathogens can be modulated by environmental conditions. In particular, adhesin expression can be modulated by iron. To determine if iron has a role in the expression of PfEMP1, we cultured IRBC in the presence of the iron chelator desferrioxamine (DFO). At 20 μM of DFO, no effect on PfEMP1 gene transcription was observed. Using Western blotting and flow cytometry, we showed that while DFO did not affect total PfEMP1 protein production, it significantly reduced the amount of protein that was detected on the cell surface. The difference in the amount of surface-expressed PfEMP1 between control and DFO-treated IRBC was detected in the early trophozoite stage (20-24 h) and persisted through the second half of the life cycle. The reduced protein expression was associated with abnormal knob formation reminiscent of that seen on the surface of IRBC containing HbC and HbS. Most importantly, these phenotypic changes were associated with decreased adhesion of DFO-treated IRBC to human microvascular endothelium in a flow chamber assay. The inhibitory effect of DFO on cytoadherence could be reversed by pre-incubating DFO with equimolar concentrations of ferric chloride. These findings strongly suggest that the trafficking of PfEMP1 to the red cell surface may be defective in an iron-depleted environment.

## 335

### HIGH POLYMORPHISM OF THE PF-SERCA IN *PLASMODIUM FALCIPARUM* FIELD ISOLATES

**Ronan Jambou**[1], Axel Martinelli[2], João Pinto[2], Simonetta Gribaldo[1], Eric Legrand[3], Makhtar Niang[4], Nimol Kim[5], Béatrice Volnay[3], Marie Thérèse Ekala[1], Christiane Bouchier[1], Thierry Fandeur[5], Pedro Berzosa[6], Isabel Dinis Ferreira[2], Cynthia Ferreira[7], Pedro Paulo Vieira[7], Maria das Graças Alecrim[7], Odile Mercereau-Puijalon[1], Pedro Cravo[2]

*[1]Institut Pasteur, Paris, France, [2]Centro de Malária e Outras Doenças Tropicais/IHMT/UEI Biologia Molecular, Lisbon, Portugal, [3]Institut Pasteur de Guyane Française, Cayenne, France, [4]Institut Pasteur de Dakar, Dakar, Senegal, [5]Institut Pasteur du Cambodge, Phnom Penh, Cambodia, [6]Centro Nacional de Medicina Tropical, Madrid, Spain, [7]Fundação de Medicina Tropical do Amazonas, Manaus, Brazil*

Artemisinin is a thapsigargin-like sesquiterpene active against multidrug resistant parasites. It targets PfSERCA, disturbing calcium homeostasis in the parasite. SERCA are P-ATPase pumps composed of a transmembrane channel and a cytosolic headpiece which regulates the binding of calcium and closing of the calcium channel. This study was conducted on a large range of field isolates to better understand genetic polymorphism of the Pf-serca gene and its relation with drug resistance. Amino-acid sequence alignment of Pf-SERCA with other proteins showed that all Pf-SERCA functional domains, as well as its transmembrane helix, are very well conserved. However, Pf-SERCA also harboured a unique area, located mainly in the head part of the protein. Additionally, we found a large sequence diversity, with 29 mutated codons and 32 SNPs from 118 isolates. The prevalence of SNPs was highest in Senegal, Brazil and French Guiana, with less mutations in Asia. Location of the SNPs in the gene supports a geographic clustering of the falciparum population, with a clear separation between Old World and New World samples. Two main ancestors were found, one common to all the samples and the other present in Africa and Asia, but not in America. A purifying selection seems to operate in French Guiana (FG) and Senegal. However, polymorphism was higher than expected by simple genetic drift. As all the isolates were collected before deployment of artesunate containing therapies, this suggests a pre-existent polymorphism driven by other genetic forces. Most SNPs were located in the falciparum-specific part of the gene, with nonsynonymous mutations located outside the functional part of the protein. However, the cation-binding area appeared under selective pressure, thus linking calcium pathways and SERCA evolution. Similarly, the S769N mutation found in FG associated with higher IC50 for arthemeter, was located close to the hinge of the head piece, and could decrease affinity for calcium as well as the efficacy of the drug. Interestingly, the prevalence of mutations described for each country in this study, and by others, is closely related to the prevalence of haemoglobinopathies in the population. These diseases are also associated with calcium disregulation in erythrocytes. These data taken together, support calcium homeostasis as the selective pressure inducing the high polymorphism of Pf-SERCA.

## 336

### FYA/FYB ANTIGENIC POLYMORPHISM SIGNIFICANTLY ALTERS BINDING OF *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN TO HUMAN ERYTHROCYTES

Jia Xianli[1], Brian Grimberg[1], Asim A. Siddiqui[1], Amy McHenry[2], Lior Greenberg[3], John H. Adams[4], Peter A. Zimmerman[1], **Christopher L. King**[1]

*[1]Case Western Reserve University, Cleveland, OH, United States, [2]University of Notre Dame, Notre Dame, IN, United States, [3]University Hospitals, Cleveland, OH, United States, [4]University of South Florida, Tampa, FL, United States*

The Duffy Blood system (Fy) comprises two polymorphic antigens, Fya and Fyb, resulting from a single amino acid substitution of Gly44Asp respectively. The N-terminal 60 amino region of the Duffy antigen is expressed on the human erythrocyte surface and is an essential receptor of *Plasmodium vivax* (Pv) and P. knowlesi for erythrocyte invasion and functions as a promiscuous chemokine receptor. The functional significance of this polymorphism has not been defined. Based on recent epidemiological reports that individuals expressing the Fyb compared to the Fya allele are more susceptible to *P. vivax* blood-stage infection, we hypothesized that this polymorphism effects binding affinity of *P. vivax* Duffy Binding Protein (PvDBP) to erythrocytes. To test this hypothesis we expressed and refolded PvDBP and measured its binding to erythrocytes by flow cytometry and in the COS cell assay. The level of PvDBP binding to erythrocytes among individuals was highly consistent with repeated sampling. We found PvDBP showed 41% greater binding to Fya-/b+, compared to Fya+/b- (P=0.0003, Mann-Whitney U-test); Fya+/b+ expressing erythrocytes showed intermediate binding. The level of Duffy antigen expression on erythrocytes, evaluated by anti-Fy6 binding, was similar among the different phenotypes and underlying genotypes. The Fya ->Fyb did not affect binding of the chemokine RANTES. Importantly, equivalent titers of artificially or naturally induced antibodies to PvDBP showed 2-fold lower binding inhibitory activity against Fya-/b+ - compared to Fya+/b expressing erythrocytes indicating more and/or higher affinity antibody is required to block binding of PvDBP to Fya-/b+ expressing erythrocytes. In conclusion these results have implications for development of a PvDBP-based vaccine and suggest that Fy allelic differences may affect erythrocyte invasion by *P. vivax* and P. knowlesi.

## 337

### GENETIC VALIDATION OF THE PLASMODIAL SURFACE ANION CHANNEL AS AN ANTIMALARIAL DRUG TARGET

**Ajay D. Pillai**, Sanjay A. Desai

*National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States*

Intraerythrocytic malaria parasites induce an unusual ion channel known as the plasmodial surface anion channel (PSAC) on their host erythrocyte membrane. PSAC has broad permeability to a diverse collection of solutes, including nutrients required for *in vitro* parasite growth. Because PSAC activity is absent from uninfected erythrocytes and because nonspecific inhibitors sterilize parasite cultures, this channel is being pursued as a target for antimalarial drug development. To more rigorously examine the suitability of this target, we identified a small molecule inhibitor having a significant (~ 200-fold) difference in efficacy against channels expressed by the Dd2 and HB3 parasite isolates. This difference in efficacy was apparent in both organic solute uptake and single PSAC patch-clamp, consistent with a single channel for transport of diverse solutes. Under optimized *in vitro* culture conditions, this inhibitor killed both isolates with IC50 values that paralleled those for inhibition of transport (0.25 ± 0.03 μM vs. 53 ± 4 μM for Dd2 and HB3 isolates, respectively). We also examined inheritance in the available progeny clones from the HB3 x Dd2 genetic cross and found a precise correlation between transport and growth inhibition by this PSAC inhibitor. These studies exclude off-target effects as the basis of parasite killing, provide insights into PSAC's biological role for the parasite, and definitively validate this ion channel as a target for antimalarial drug discovery and development.

## 338

### A UNIVERSAL APPROACH TO EXPRESS DIVERSE *P. FALCIPARUM* ENZYMES IN FUNCTIONAL FORM

**Devaraja G. Mudeppa**, Pradipsinh K. Rathod

*University of Washington, Seattle, WA, United States*

A severe limitation to the development malaria therapeutics is the low yield of functional recombinant proteins. Previous genome-wide attempts to express *P. falciparum* proteins using conventional, cell-based, heterologous expression technologies failed at rates of 93 and 80% (MBP (2006) 148, 144-160; MBP (2007) 151, 100-110). We previously

demonstrated the utility of a wheat germ, cell-free protein expression system to overproduce malaria dihydrofolate reductase-thymidylate synthase, a well-known, challenging case (MBP (2007) 151, 216-219). To generate protocols with universal utility, we selected 14 test case enzymes from *P. falciparum* genome that are directly or indirectly involved in nucleic acid metabolism, an area of interest to our lab. The selected genes range in size from 19 to 169 kDa. Many of these enzymes have a history of failure to express in standard cell-based expression systems. All 14 genes were reverse amplified from total RNA to minimize annotation problems and were directly cloned into a TA-based cell-free expression vector with promoters and RBS orthogonal to *E. coli*. After *in vitro* transcription and translation, autoradiography demonstrated synthesis of single products of expected mass. Scaled-up expression routinely produced up to 250 ug of soluble protein per ml of lysate and, for the first time, all 14 gene products were enzymatically active. This ability to produce malarial proteins with a high success rate, without codon optimization or genetic selection in *E. coli*, opens up exciting avenues to study previously inaccessible malarial gene products.

## 339

### COMPARATIVE ANALYSIS OF SECRETED PROTEINS IN APICOMPLEXAN PARASITES

**Rays H. Jiang,** Daniel E. Neafsey

*The Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge, MA, United States*

Apicomplexa are comprised of a diverse group of intracellular parasites. Classes such as Haemosporea, Coccidea, and Piroplasmea cause medically and economically important diseases of humans and domestic animals worldwide. Amongst these, Plasmodium is the causal agent of the devastating diseases of malaria. To achieve their obligate style of intracellular parasitism, Apicomplexa secrete a large array of proteins to colonize and modulate their host cells. We have computationally analyzed the secreted proteins of different species across Apicomplexa by using signal peptide prediction, trans-membrane domain identification, and hidden Markov model (HMM) search of the host targeting motifs. An estimated 10-20% of Apicomplexan proteomes is dedicated to be secreted and deployed on the parasite surface, host cellular environment and host cellular surface. For different family of proteins, different rate of nucleotide substitution and lineage expansion are found. These evolutionary patterns may reflect the different aspects of pathogenesis such as attachment to and invasion of host cell, survival within the parasitophorous vacuole (PV), manipulation of host cell metabolism and evasion of host immune responses.

## 340

### GENETIC MAPPING IN TWO *PLASMODIUM FALCIPARUM* CROSSES IDENTIFIES A LOCUS ENCODING THE PLASMODIAL SURFACE ANION CHANNEL

**Abdullah A. Bokhari**, Sanjay A. Desai

*National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States*

Malaria parasites increase their host erythrocyte's permeability to diverse solutes, including nutrients required for parasite growth. The plasmodial surface anion channel (PSAC) appears to mediate these permeability changes and is an important new antimalarial drug target. Although PSAC's functional properties have been well studied, its genetic basis remains unknown. Here, we performed high-throughput screens for PSAC antagonists using four divergent laboratory isolates. Although most identified inhibitors were equally effective against each isolate, a small number had inhibitory affinities that differed significantly between isolates. Two unrelated inhibitors exhibited differences in PSAC affinities between parental isolates of the HB3 x Dd2 and 7G8 x GB4 genetic crosses, permitting examination of inheritance in 64 independent progeny clones from these two crosses. Each progeny clone segregated into one of the two parental phenotypes with approximately half resembling each parent, suggesting Mendelian inheritance of PSAC inhibitory affinity. In each cross, quantitative trait locus (QTL) mapping yielded a single locus with high confidence (LOD scores > 20 each). The loci identified in these two independent genetic crosses were identical, suggesting that one or more genes in their consensus region are primarily responsible for inheritance of PSAC activity. Identification of PSAC's gene(s) should permit heterologous expression of this unusual parasite channel, directed molecular studies of how the channel selects and transports solutes, and more rigorous validation of PSAC as a therapeutic target.

## 341

### MAPPING THE GENETIC CONTROL OF THE GLOBAL METABOLITE PROFILE IN *PLASMODIUM FALCIPARUM*

**Mark A. Wacker**[1], Kellen A. Olszewski[2], Asako Tan[1], Geoffrey H. Siwo[1], Joshua D. Rabinowitz[3], Maneul Llinas[2], Michael T. Ferdig[1]

*[1]Eck Institute for Global Health, University of Notre Dame, Notre Dame, IN, United States, [2]Department of Molecular Biology and Lewis-Singler Institute for Integrative Genomics, Princeton University, Princeton, NJ, United States, [3]Department of Chemistry and Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ, United States*

The major obstacle to existing malaria control strategies is the ongoing emergence of parasites resistant to the few remaining effective antimalarial drugs. Our general understanding of drug resistance physiology is limited, and the drug development pipeline has not fully exploited the vast information emerging from whole-genome technologies. Global metabolite analysis provides a direct and unbiased view of the biochemical consequences of genetic variation. Using a mass spectrometry-based approach, we have assayed global metabolite levels in the parents and progeny of a *Plasmodium falciparum* genetic cross (HB3 vs. Dd2). More than 12,000 mass peaks were measured from extracts derived from highly synchronous parasite cultures. These mass peaks, indicating inherited levels of different metabolites, were treated as phenotypes for quantitative trait loci (QTL) mapping to localize the underlying genetic contributors. For this analysis, all mass peaks were incorporated into the analysis, whether or not the compound identity was known, and more than 500 of the mass peaks exhibited a significant QTL. We find that compound QTL are not randomly distributed across the genome but instead cluster into several hot spots that play a major role in regulating parasite metabolism. For some known compounds, it is possible to directly identify genes in the locus that may modulate a biochemical pathway; in other cases, locus information may be used to infer compound identity. Several of the most significant linkages map to regions of the *P. falciparum* genome previously shown to determine drug sensitivities inherited in these progeny. Further investigation and characterization of these metabolites will elucidate the role these loci play in drug resistance and may serve to identify possible novel targets for intervention.

## 342

### IMMUNE CONSTRAINTS ON PARASITEMIA AND GAMETOCYTEMIA IN MALARIA: INSIGHT FROM NUMERICAL STUDIES

**Philip G. McQueen**, F. Ellis McKenzie

*National Institutes of Health, Bethesda, MD, United States*

Plasmodium infections trigger patient-specific complex immune reactions and strong erythropoietic responses. To elucidate the potential consequences of these host responses upon the conversion of the asexual forms to gametocytes (which transmit the disease when taken up by mosquitoes), we developed differential equation models of Plasmodium-red blood cell dynamics modulated by host erythropoietic and immune reactions. Model immune responses had two components: a quickly acting but quickly decaying response to emulate the innate system reactions observed clinically, and a slower acting, long term response to emulate

antibody responses. In additions, the models were tailored to the specific life cycles of *P. falciparum* and *P. vivax*. We considered several parasite developmental stages as targets for each type of immune response, and we varied over wide ranges the parameters that specify both sensitivity to parasite and quickness of removal of parasite. Also, we considered different plausible mechanisms for triggering gametocyte production. We found that (1) immune conditions which lead chronic parasitemia of the asexual forms allow chronic gametocytemia as well. (2) If host immune responses fail to quickly control a *P. falciparum* infection, a rapid onset of fatal anemia can actually limit production of the mature transmissible gametocytes and reduce the period of infectiousness. (3) On the other hand, host failure to control *P. vivax* can lead to fatal anemia and high gametocytemia both, due to the slower onset of severe anemia in *P. vivax* infection. (4) For a wide variety of immune responses, parasitemia and gametocytemia levels have a nonlinear dependence on the sharpness of the transitions between intra-RBC phase and free merozoites. Clinically, most patients mount an immune response that controls their *P. vivax* infection before the fatal anemia occurs. However, recent clinical reports of *P. vivax* causing *P. falciparum*-like illness suggest that failure to control *P. vivax* can indeed to severe disease for the host, in line with our predictions.

## 343

### ABO POLYMORPHISM AND *PLASMODIUM FALCIPARUM* MALARIA: ENHANCED PHAGOCYTOSIS OF INFECTED O ERYTHROCYTES

**Kayla T. Wolofsky**[1], Kodjo Ayi[1], W. Conrad Liles[1], Christine M. Cserti-Gazdewich[2], Kevin C. Kain[1]

*[1]McLaughlin-Rotman Centre for Global Health, University Health Network, University of Toronto, Toronto, ON, Canada, [2]Blood Transfusion Laboratory, Toronto General Hospital, Department of Laboratory Hematology, University of Toronto, Toronto, ON, Canada*

Malaria has been a major selective force on red blood cell (RBC) polymorphisms that confer protection to severe disease. Several lines of evidence suggest that the outcome of *Plasmodium falciparum* infection may also be influenced by ABO blood group antigens. Blood type O predominates in malaria endemic regions and has been associated with protection from developing severe and complicated malaria. Although the molecular details of protection have not been fully elucidated, previous studies have demonstrated reduced rosetting in type O RBCs. Based on observations showing that enhanced phagocytosis of infected RBCs occurs with other RBC polymorphisms associated with protection, we hypothesized that infected type O RBCs may be more efficiently cleared by the innate immune system than type A RBCS. Here we show that primary human macrophages phagocytosed *P. falciparum*-infected type O RBCs more avidly than infected type A RBCs ($p<0.001$). Phagocytosis was independent of macrophage donor ABO blood type. Furthermore, we developed an *in vivo* phagocytosis assay and demonstrate that murine intra-peritoneal monocytes phagocytosed infected O RBCs more avidly than infected A RBCs *in vivo* ($p=0.008$). These findings led us to investigate whether the number and structure of cell surface antigens influenced the uptake of parasitized erythrocytes. The phagocytic uptake of infected A2 RBCs was shown to be intermediate between O and A1 infected RBCs. To confirm our results we have extended these studies to include competition phagocytic assays using synthetic blood group antigens to inhibit uptake and transformation experiments whereby A and B RBCs are converted to "O" RBCs via enzymatic cleavage of blood group antigens. Collectively, our data suggest that type O individuals have more proficient clearance of infected RBCs. If these observations extend to *P. falciparum* infection *in vivo*, then this may contribute to an overall decrease in parasite burden and a reduction in the number of infected erythrocytes available to bind within the micro-vascular beds of vital organs. Enhanced clearance of infected RBCs may represent an additional putative mechanism by which blood type O may contribute to protection against severe malaria.

## 344

### STRUCTURAL AND BIOCHEMICAL CHARACTERIZATION OF THE BINDING REGION OF *PLASMODIUM FALCIPARUM* VAR2CSA DBL3X WITH CHONDROITIN SULFATE A

**Kavita Singh**[1], Rossitza K. Gitti[2], Apostolos G. Gittis[1], Phuc G. Nguyen[1], Michael J. Mohan[1], D. Channe Gowda[1], D. Channe Gowda[3], Gregory Tullo[1], Hong Zhou[1], Rick Fairhurst[1], Carole Long[1], David N. Garboczi[1]

*[1]National Institute of Allergy and Infectious Diseases/National Institutes of Health, Rockville, MD, United States, [2]ECB Forensic Analytical Branch, Aberdeen, MD, United States, [3]Pennsylvania State University College of Medicine, Hershey, PA, United States*

The pathogenesis of malaria in pregnant women is primarily due to the accumulation of *Plasmodium falciparum*-infected erythrocytes in the placenta. DBL3x domains of VAR2CSA, a parasite-encoded member of the PfEMP1 family of proteins, are expressed on the infected erythrocyte surface where they contribute to the binding of parasitized erythrocytes to chondroitin sulfate A (CSA) in the placenta. Recently we have determined the crystal structure of a VAR2CSA DBL3x domain in complex with CSA and identified the location of the CSA binding site. We have developed polyclonal rabbit and mouse monoclonal antibodies to both the DBL3x domain and its CSA-binding subdomain. Flow cytometry shows that IgG purified from rabbit polyclonal antiserum bind to *P. falciparum*-infected erythrocytes selected *in vitro* for adherence to CSA. Antisera obtained from Malian women, but few Malian men, recognize the DBL3x protein by ELISA, as would be anticipated with an antigen primarily associated with malaria in pregnancy. Together these preliminary results support the conclusion that the recombinant DBL3x domain and its CSA-binding subdomain are structurally similar to the DBL3x natively expressed on the surface of CSA-adherent parasitized erythrocytes. We have further analyzed and characterized the CSA-binding region as well as ascertained the contribution of charged residues involved in CSA binding and their functional studies by using site-directed mutagenesis, immunological, biochemical and biophysical methods

## 345

### IMMEDIATE NEUROPSYCHOLOGICAL AND BEHAVIORAL BENEFITS OF COMPUTERIZED COGNITIVE REHABILITATION IN UGANDAN PEDIATRIC CEREBRAL MALARIA SURVIVORS

**Paul Bangirana**[1], Bruno Giordani[2], Chandy C. John[3], Connie Page[4], Robert O. Opika[1], Michael J. Boivin[4]

*[1]Makerere University, Kampala, Uganda, [2]University of Michigan, Ann Arbor, MI, United States, [3]University of Minnesota, Minneapolis, MN, United States, [4]Michigan State University, East Lansing, MI, United States*

Our earlier studies on Ugandan children surviving cerebral malaria showed cognitive deficits mainly in attention and memory. We now present the first study in sub-Saharan Africa to investigate the feasibility and potential benefits of computerized cognitive rehabilitation training on neuropsychological and behavioural functioning of children surviving cerebral malaria. A randomized trial in which 65 children admitted 45 months earlier with cerebral malaria were recruited at Mulago Hospital, Kampala, Uganda. For eight weeks, 32 of the children received weekly training sessions using Captain's Log cognitive training software and the other 33 were assigned to a non treatment condition. Pre- and post-intervention assessments were completed using CogState, a computerized neuropsychological battery, measuring Visuomotor Processing Speed, Working Memory, Learning, Attention and Psychomotor Speed and the Child Behavior Checklist measuring Internalising Problems, Externalising Problems and Total Problems. Pre-intervention scores were similar between both groups. Treatment effects were observed on Visual Spatial Processing Speed (group effect (standard error) 0.14 (0.03); $p< 0.001$); on a Working Memory and Learning task (0.08 (0.02); $p< 0.001$), Psychomotor Speed (0.14 (0.07); $p= 0.04$) and on Internalising Problems (-3.80 (1.56); $p= 0.02$) after controlling for age, sex, school grade, quality of the home

environment and weight for age z scores. Similar treatment effects were observed when no adjusts for the above covariates were made. In conclusion, computerized cognitive training long after the cerebral malaria episode has immediate benefit on some neuropsychological and behavioral functions in African children. The long-term benefit of this intervention needs to be investigated.

## 346

### ANTIGEN SPECIFICITY OF CD23 (FCEPSILONRII)-BOUND IGE MODULATES B CELL RESPONSES: IMPLICATIONS FOR IMMUNITY TO SCHISTSOMIASIS

**Qyana Kelly Griffith**[1], YanMei Liang[1], Taslima Shaikh[2], Helmut Haas[3], Daniel Onguru[4], Pauline Mwinzi[4], Lisa Ganley-Leal[1]

*[1]Boston University School of Medicine, Boston, MA, United States, [2]Boston University, Boston, MA, United States, [3]Research Center Borstel, Borstel, Germany, [4]Kenya Medical Research Institute, Kisumu, Kenya*

Human schistosomiasis affects over 207 million individuals and is a significant cause of morbidity in the developing world. Previous data from multiple laboratories suggest that high levels of schistosome-specific IgE are associated with resistance to schistosomiasis. Furthermore, we have demonstrated that CD23 (the low affinity IgE receptor) expression by circulating B cells is also correlated with resistance to infection. However little is known about the role of CD23-bound IgE in the immune response and how host-parasite interactions might affect this process. Human B cells circulate pre-loaded with IgE raising the questions as to how a B cell discriminates between all the antigens it can potentially capture and how would IgE of other specificities affect this response, especially in regions where multiple endemic pathogens induce hyper-IgE responses. We tested how CD23-bound IgE might influence B cell responses to antigen using our model system. Dust mite-specific IgE or polyclonal IgE pooled from blood donors was bound to nascent IL-4 induced CD23 on tonsil B cells. B cell activation was measured via phospho-flow upon the introduction of dust mite antigen. We found that the threshold of antigen-specific IgE affects B cell activation in response to antigen, not only via CD23-bound IgE, but also through the B cell receptor. These results suggest that pre-existing IgE specific for other pathogens will affect a B cell's response to a new infection. We next evaluated how a glycoprotein, IPSE/alpha-1, which binds to the Fc portion of IgE and affects other IgE-mediated responses, would influence B cell activation. Blood and tonsil IgE+CD23+ B cells were stimulated with anti-IgE to cross-link pre-existing CD23-bound IgE in the presence of IPSE/alpha-1. Remarkably, IPSE/alpha-1 specifically inhibited anti-IgE activation through specific phosphorylation events. We conclude that CD23-bound IgE plays an important role in human B cell activation and pre-existing IgE levels and specificities will likely affect an individual's immune response to schistosome infection. IPSE/alpha-1 is likely produced by schistosomes as an immuno-evasive tactic to overcome specific B cell signaling pathways.

## 347

### IN UTERO EXPOSURE TO MATERNAL SCHISTOSOMIASIS MODULATES ACUTE AND MEMORY CELLULAR AND HUMORAL IMMUNE RESPONSES OF OFFSPRING

**Allison C. Brown**, William J. Moss

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

Observational studies in humans indicate that in utero exposure to maternal helminth infection modulates immune responses to vaccines in their children, although the immunological mechanisms have not been identified. Helminth antigens cross the placenta and transplacental transfer of cytokines, antibodies or maternal lymphocytes may play a role in immune modulation. To investigate the mechanisms by which maternal helminth infection modulates immune responses to vaccines in their offspring, we developed a murine model of in utero exposure to maternal schistosomiasis. Balb/c dams were exposed to ~20 live Schistosoma mansoni cercariae and mated with Balb/c males 6 weeks after infection. Offspring of infected and uninfected dams received an alphavirus replicon expressing measles virus H protein (VCR-H) or a PBS control vaccine at 4 weeks of age, and were sacrificed at 2 or 6 weeks after vaccination. Using ELISPOT assays to measure splenocyte responses to bystander and vaccine-specific antigens, a dose response effect was observed between maternal egg burden and IL-4 and IFN-γ cytokine production at 2 and 6 weeks post-vaccination. Acute responses at 2 weeks post-vaccination were directly correlated with maternal egg burden. In contrast, memory responses at 6 weeks were inversely correlated with maternal egg burden, suggesting that in utero exposure results in short-term activation but long-term suppression of T cell responses after vaccination. Acute IL-4 and IFN- γ production was split respectively between CD4+ and CD8+ T cells by intracellular cytokine staining, but memory CD8+ T cells were the primary producers of both IL-4 and IFN-γ. Maternal egg burden and longer duration of infection in dams were associated with CD4+ T cell populations with regulatory T cell profiles (Foxp3+ or CD25+Foxp3+) in their offspring, a potential mechanism of long-term immune suppression following in utero exposure to maternal schistosomiais. Among VCR-H vaccinated offspring, the IgG2a to IgG1 ratio increased with maternal egg exposure, consistent with enhanced Th1 skewing, but total vaccine-specific IgG titers decreased with increasing maternal egg exposure. These observations demonstrate that in utero exposure to maternal infection modulates both the acute and memory cellular and humoral immune responses of their offspring, and may decrease vaccine efficacy in offspring.

## 348

### SCHISTOSOMES TARGET HUMAN CD23-MEDIATED IMMUNITY IN IMMUNO-EVASIVE TACTICS

Qyana Griffith[1], YanMei Liang[1], Ashley Cruz[1], Helmut Haas[2], **Lisa Ganley-Leal**[1]

*[1]Boston University School of Medicine, Boston, MA, United States, [2]Research Centre Borstel, Borstel, Germany*

We previously reported that CD23 expression by human circulating B cells correlates with resistance to reinfection with schistosomes. CD23 is the low affinity IgE receptor involved in IgE regulation and antigen presentation by B cells. CD23-bound IgE is cross-linked by antigen to induce B cell activation. CD23 may also become soluble following host-mediated cleavage to affect many aspects of immunity. Our new data indicate that schistosomes target CD23-mediated immunity via several mechanisms. First, we have found that all three mammalian life stages of schistosomes cleave surface CD23 from human B cells. This cleavage was inhibited by the cysteine protease inhibitor, leupeptin, suggesting that schistosomal calpain is involved in reducing surface CD23. Second, the resulting soluble CD23 from schistosomal-mediated cleavage was distinctive from host-mediated sCD23 and demonstrated the potential to bind and sequester IgE, possibly abrogating IgE-mediated immunity via the high affinity IgE receptor. Third, a glyco-protein, IPSE/alpha-1, produced by schistosome eggs influenced CD23-bound IgE mediated B cell activation likely through binding to the Fc portion of IgE. And finally, B cells were induced to express an alternative CD23 isoform, CD23b, in the presence of schistosome antigens which we predict will further inhibit protective B cell CD23 responses. IgE has been observed to be associated with resistance to schistosomes for many years though the mechanism by which IgE confers protection continues to elude us. Our work sheds light on the host-parasite interactions between IgE and its receptors in human schistosomiasis and thus may lead us to rational vaccine candidates and development.

## 349

### SUBVERSION OF INNATE IMMUNE SIGNALS BY *SCHISTOSOMA MANSONI* PERMITS WORM DEVELOPMENT

**Diana K. Riner,** Sean K. Maynard, Stephen J. Davies

*Uniformed Services University of the Health Sciences, Bethesda, MD, United States*

Helminth blood flukes of the genus *Schistosoma* infect over 200 million people world wide. Perhaps as a result of extensive host-parasite co-evolution, *S. mansoni* has evolved to exploit host immune factors as signals to coordinate its own development within the host. Worms fail to develop normally in $RAG^{-/-}$ mice that lack all T and B cells, while development is restored when $CD4^+$ T cells are transferred into $RAG^{-/-}$ mice, suggesting that $CD4^+$ T cells play a central role in regulating parasite development. However, recent findings suggest the role of $CD4^+$ T cells in this process is indirect, being limited to provision of non-cognate T cell help for innate responses which, in turn, facilitate parasite development. In support of this hypothesis, we have found that administration of LPS to $RAG^{-/-}$ mice, in the absence of $CD4^+$ T cells, also restores worm development, indicating that innate immune signals are sufficient for parasite development to proceed normally. LPS, a pathogen-associated molecular pattern (PAMP), activates TLR-4, resulting in signaling through both MyD88-dependent and TRIF-dependent pathways. Interestingly, specific stimulation of TRIF signaling using monophosphoryl lipid A (MPLA) or poly-IC failed to restore worm development in $RAG^{-/-}$ mice, as did administration of exogenous IFN-$\alpha$, suggesting that worm development is not dependent on TRIF-mediated induction of type I interferon expression. Our current research efforts are therefore focused on dissecting the MyD88-linked signaling events that influence schistosome development. Elucidation of the innate immune signals that control schistosome development could provide leads for the development of new drug targets and vaccine strategies.

## 350

### REGULATION OF INNATE IMMUNITY TO LEISHMANIA INFECTION BY TYPE I IFN SIGNALING

**Lijun Xin**, Diego A. Vargas-Inchaustegui, Jiaren Sun, Lynn Soong

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

Type I IFNs exert diverse effector/regulatory functions in innate and adaptive immune responses to viral and non-viral infections; however, their roles in parasitic infections are less clear. In murine models of *Leishmania* infection, it has been reported that parasite-induced type I IFNs are critical for NO-dependent disease control, and that administration of IFN-β has a dose-dependent protective effect in *L. major*-infected mice. Surprisingly, we found that compared to WT controls, IFNAR-/- mice developed significantly smaller lesions and reduced Ag-specific immune responses following infection with *L. amazonensis* (*La*). The marked reduction in tissue parasite loads even at 3 days of infection in IFNAR-/- mice suggested the possibility of neutrophil-mediated parasite clearance. This hypothesis was supported by *in vitro* and *in vivo* studies. First, IFNAR-/- mice showed sustained infiltration of neutrophils, but limited recruitment of CD11b+Ly6C+ inflammatory monocytes, in inflamed ear tissues at day 7 and in inflamed peritoneal cavity at day 2. Second, while macrophages responded comparably to parasites, the interactions between macrophages and IFNAR-/- neutrophils (but not WT or STAT1-/- neutrophils) greatly enhanced parasite killing. Third, in comparison to WT and STAT1-/- counterparts, IFNAR-/- neutrophils had significantly higher rates of spontaneous and infection-induced apoptosis and released higher levels of neutrophil elastase and myeloperoxidase. Finally, while co-injection of IFNAR-/- neutrophils with parasites reduced parasite survival, co-injection of WT neutrophils with parasites or adoptively transfer of WT neutrophils into IFNAR-/- mice markedly enhanced tissue parasite loads. This study indicates an important role for type I IFNs in regulating neutrophil turnover and *Leishmania* infection and provides new information on innate immunity to protozoan parasites.

## 351

### VIRAL DETERMINANTS OF DENGUE VIRUS FITNESS AND VIRULENCE REVEALED IN THE EVOLUTION OF DENGUE VIRUS SEROTYPE 2 IN NICARAGUA

**Molly OhAinle**[1], Matthew R. Henn[2], Aubree Gordon[1], Tangni Gomez[3], Yolanda Tellez[3], Saira Saborío[3], Bruce Birren[2], Angel Balmaseda[3], Eva Harris[1]

*[1]Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, Berkeley, CA, United States, [2]Broad Institute, Cambridge, MA, United States, [3]Departamento de Virología, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud, Managua, Nicaragua*

Four serotypes of dengue virus (DENV-1-4) circulate globally, causing more human illness than any other arbovirus. DENV infection results in dengue fever, a debilitating acute febrile illness, or the more severe, life-threatening dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). The genetic make-up of DENV, in addition to host immune status and genetic variables, contributes to disease severity. Viral evolution may result in increased DENV replication in host cells or altered sensitivity to neutralization or enhancement by host antibody responses. Here we describe an increase in dengue disease severity between 2005 and 2006-7 in two independent studies in Managua, Nicaragua, that cannot be explained by clinical/epidemiological variables or by introduction of a new DENV serotype or strain. Whole-genome sequencing of viruses isolated from patient sera reveals the evolution of a new clade of Asian-American genotype DENV-2 between 2005 and 2006-7 in Nicaragua ($p<0.0001$). Consistent with a model of replacement of the circulating DENV-2 clade by a more fit and perhaps more virulent clade, the new DENV-2 clade correlates with DHF/DSS ($p<0.05$). The hypothesis of increased fitness/virulence is being tested *in vitro* by infection of cell lines and primary cells of monocytic lineage with primary viral isolates and infectious clones representing both Nicaraguan DENV-2 clades. Infection assays are being performed independent of Ab-mediated entry in both U937/DC-SIGN and primary human dendritic cells. To ask if increased fitness occurs in the context of secondary infection, infectivity of viruses from both clades is being tested in THP-1 cells through Ab-mediated infection. Further, the relative fitness of both viral clades is being examined in the context of the host immune response by measuring the relative neutralization of the two clades by pre-existing heterotypic antibodies using patient sera from the Nicaraguan children from whom the viruses in question were isolated. Mutations identified through phylogenetic analyses, including several changes in E, NS1 and NS5 proteins as well as in the 5′ and 3′ UTRs are being assayed for effects on viral replication. The observed increase in disease severity associated with evolution within a single genotype of DENV-2 over a limited time span and geographic range provides a unique opportunity to explore the viral determinants of DENV fitness and virulence.

## 352

### ASSESSING THE ROLE OF HUMAN MOVEMENT IN THE TRANSMISSION DYNAMICS OF DENGUE VIRUS IN IQUITOS, PERU

**Steven T. Stoddard**[1], Amy C. Morrison[1], Brett M. Forshey[1], Valerie Paz Soldan[2], Helvio Astete[1], John P. Elder[3], Gonzalo Vasquez-Prokopec[4], Uriel Kitron[4], Tadeusz J. Kochel[5], Thomas W. Scott[1]

*[1]University Of California, Davis, Davis, CA, United States, [2]Tulane University, New Orleans, LA, United States, [3]San Diego State University, San Diego, CA, United States, [4]Emory University, Atlanta, GA, United States, [5]Naval Medical Research Center Detachment, Lima, Peru*

Entomological risk of infection with dengue virus is usually evaluated at the household level, even though individual human hosts move frequently during the day when the vector, *Aedes aegypti*, is active. With a simple model we illustrate that variation in exposure to infective mosquitoes due