same tick vector and an overlapping community of vertebrate reservoir hosts, expansion of babesiosis has markedly lagged that of Lyme and is endemic in a much smaller range. Invasion of tick-borne pathogens is a product of local, small-mammal mediated spread and long-distance, bird-mediated spread. However, the relative contribution of different hosts to pathogen dispersal and the factors driving the differential expansion trajectories for the two pathogens remain unknown. We use a hierarchical Bayesian framework for testing mechanistic hypotheses that describe the spatio-temporal distribution of human cases of each disease across the Northeast. The model is parameterized with data compiled from state and county health departments from 1984-2011 for Lyme disease and from 1990-2011 for babesiosis. Three model structures are developed for comparison. Specifically, we examine the relative importance of local versus long-distance spread processes for predicting invasion dynamics of the two diseases. We also identify climate, landscape and vector biology factors significant in predicting pathogen diffusion. The best-fitting model structure included both spatially variable local diffusion and long-distance spread. Diffusion of babesiosis was found to be consistently slower than that of Lyme across the Northeastern emergence focus. This data-driven mechanistic framework is a simple but accurate approach to studying the invasion dynamics of pathogens maintained in complex ecological cycles such as the tick-borne infections presented here. Our method can be used to predict sites of probable invasion, thus identifying spatial targets for enhanced surveillance and control measures.

749

PHLEBOTOMUS ORIENTALIS SALIVARY ANTIGENS - IDENTIFICATION, CHARACTERIZATION AND EXPRESSION

Michal Sima[1], Michaela Vlková[1], Tatiana Košťálová[1], Aysheshm Kassahun[1], Carla Maia[1], Ryan C. Jochim[2], Jesus G. Valenzuela[2], Teshome Gebre-Michael[3], Asrat Hailu[3], Alon Warburg[4], Gad Baneth[4], Iva Kolářová[1], Petr Volf[1]

[1]Charles University in Prague, Faculty of Science, Prague, Czech Republic, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [3]Addis Ababa University, Addis Ababa, Ethiopia, [4]Hebrew University of Jerusalem, Jerusalem, Israel

Sand flies (*Diptera: Phlebotominae*) are vectors of *Leishmania* (Trypanosomatidae), the causative agents of cutaneous and visceral leishmaniasis. During the blood feeding, sand fly females inject saliva into the host skin to overcome host haemostatic mechanism. Repeated exposures to sand fly saliva elicit anti-saliva antibodies that could be used in epidemiological studies as a marker of exposure to assess the risk of *Leishmania* transmission and the effectiveness of anti-vector campaigns. The anti-saliva immunity has been also shown to protect the host from *Leishmania* infection, thus salivary proteins are considered as candidates for transmission blocking vaccine. The main aim of this study was to characterize and express salivary gland antigens of *Phlebotomus orientalis*, the important vector of life-threatening visceral leishmaniasis in East Africa. The major antigens were determined by SDS-PAGE and immunoblot using antibodies from dogs and humans repeatedly bitten by this sand fly species.. Based on the cDNA library and mass spektrometry (MALDI TOF-TOF), eight antigens from five different protein families were identified. All of these proteins with molecular weight ranging from 26 kDa to 42 kDa are antigenic for dogs but only four (apyrase, yellow-related protein, antigen 5-related protein, D7-related protein) are antigenic for humans. These four antigens were expressed in the bacterial expression system. Recombinant products will be compared in their ability to bind specific antibodies in sera from hosts repeatedly bitten by *P. orientalis* to select candidate antigen(s) usefull for larger epidemiological studies.

750

EFFECTS OF FOREST PATCH SIZE ON ABUNDANCE OF DEER TICKS (*IXODES SCAPULARIS*) AND RECOGNITION OF RED-BACKED VOLES AS MAJOR RESERVOIRS OF *BORRELIA* AND *ANAPLASMA* IN GRAND FORKS COUNTY, NORTH DAKOTA USA

Nathan M. Russart, Jefferson A. Vaughan

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

Geographic range of the deer tick, *Ixodes scapularis*, has expanded in recent years. Deer ticks are forest ticks and historically considered not to occur as far west as North Dakota. However, a statewide survey conducted in 2010 found breeding populations of deer ticks in the Northeast region of the state. This region is under intense agricultural production but within the expanse of field crops there are islands of forested areas. In 2012 six forested areas in Grand Forks County, ranging in size from 7 to 349 hectares, were surveyed for deer ticks. Ticks were collected via dragging and from small mammals. There was a significant positive correlation between forested patch size and 1) abundance of questing adults and 2) intensity of larval ticks on infested hosts. Of 1,036 deer ticks collected, the vast majority (98%) were found only at the two largest sites. However, there was no significant difference among sites in the total abundance of small mammals (mean=0.25 per trap-night), suggesting that all the forested patches regardless of their size, had sufficiently abundant hosts to sustain tick populations. The small mammal fauna at the largest forest patch (n=69) was comprised mostly of *Peromyscus* (38%) and red-back voles, *Myodes gapperi* (58%). Although the prevalence of ectoparasitism for *Peromyscus* mice (88%) was significantly greater than for *M. gapperi* voles (60%), when engorged larval ticks were detached from the rodents and assayed for pathogens (i.e., xenodiagnoses), there were no significant differences in xenopositivity between infested *Peromyscus* mice and *M. gapperi* voles for either *Borrelia burgdorferi* (overall 6% xeno-positive animals) *or Anaplasma phagocytophilum* (overall 6% xeno-positive animals). Deer ticks have become established in discreet foci within northeastern North Dakota and the role of *M. gapperi* voles as reservoirs of Lyme disease and anaplasmosis in this region warrants closer scrutiny.

751

ENTEROBIUS VERMICULARIS: A FIVE YEAR EXPERIENCE FROM AN INNER CITY HOSPITAL IN THE BRONX

Neville Jadeja, Christina Coyle, Herbert Tanowitz

Jacobi Medical Center, New York, NY, United States

Enterobius vermicularis (Pinworm or oxyuriasis) is an intestinal nematode infection commonly reported in school-age children in the United States. Reports of pinworm in the immigrant populations are emerging in the literature. Jacobi Medical Center, located in the Bronx, serves a large immigrant population. Clinical presentation, treatment and country of origin are described in patients with *E. vermicularis* infections presenting over a 5 year period to our institution. Twenty two patients were identified. Ten males (46%) were seen with mean age 17 (SD$\pm$ 17). Of these patients 20 (91%) were immigrants and of these, 6 (30%) patients gave a history of recent travel history to visit friends and family. The majority of patients seen were from Albania (9, 41%), other countries included Yemen (4, 18.2%), Morocco (4, 18.2%), Mexico (1, 5%), Pakistan (1, 5%) and Ecuador (1, 5%). Twenty patients (91%) were symptomatic. Pruritis ani was reported in 11 (50%) patients. Work-up was initiated for nonspecific abdominal pain in 8 (36%) patients and 4 (18%) patients were evaluated for enuresis. The scotch- tape was positive in 20 patients (91%). Of the 18 stools examined for ova and parasites, 4 (22%) revealed pinworm. Eighteen (82%)patients had other intestinal parasites detected on stool exam. *Dientamoeba fragilis* was found in 8 (36%) patients. The mean absolute eosinophil count was 0.5 (SD$\pm$ 0.5). Six (27%) patients had past history of pinworms which may represent recurrent infections, athough treatment failure cannot be excluded. Ten (50%) had a family members infected. Albendazole was administered to

our patients. The diagnosis is often missed or delayed because of the poor sensitivity of routine stool examination and a lack of awareness amongst primary care providers. Patients with *D. fragilis* identified in their stool should be screened for pinworm. Other members of the same household should be examined for pinworm. *E. vermicularis* needs to be considered in immigrant populations.

752

INTESTINAL PARASITES AMONG ACTIVE-DUTY MILITARY PERSONNEL IN THE PERUVIAN AMAZON

Maria S. Gallardo[1], Marilhia Cornejo[1], George Vasquez[1], Renato Errea[1], Jorge Urquiaga[1], Diego Montoya[1], Rodrigo Zamudio[1], Marco Canales[2], Angelica Terashima[2], Luis A. Marcos[3], **Jorge D. Machicado**[4]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Instituto de Medicina Tropical Alexander von Humboldt, Lima, Peru, [3]Forrest General Hospital, Hattiesburg, MS, United States, [4]University of Texas Health Science Center, Houston, TX, United States

Active-duty military personnel are exposed to soil-transmitted helminth (STH) infections when serving in highly endemic areas such as the Peruvian Amazon. Few studies have described the prevalence of STH and Strongyloides sterocoralis infection in this population. The aim of this study was to describe the prevalence of intestinal parasites among active-duty military personnel at the Peruvian Amazon basin. A descriptive observational study was carried out in a local army base of the Peruvian rainforest during December, 2012. We excluded individuals who received anthelminthic therapy in the last 3 months. One fresh stool sample was obtained from each participant and evaluated within 24 hours by the direct smear, spontaneous sedimentation in tube technique (SSTT) and modified Baermann's technique (MBT). Among 104 participants (age range, 15-34 years), 34% were infected by one parasite, whereas 57% had two or more. The most common helminths were hookworms (47%), S. stercoralis (29%), Ascaris lumbricoides (28%) and *Trichuris trichiura* (18%). The most common protozoa were *Blastocystis spp.* (57%) and *Giardia lamblia* (17%). The SSTT was the most sensitive test to detect helminthes and protozoa ($p<0.01$). MBT was more sensitive than SSTT to detect larvae of *S. stercoralis* ($p=0.04$). S. stercoralis was always associated with another parasite, and multiple regression analysis revealed a significant association to hookworm (OR = 3.88, 95% CI = 1.1-13.3), *A. lumbricoides* (OR = 3.98, 95% CI = 1.1-13.5) and *G. lamblia* (OR = 11.8, 95% CI = 1.59-86.8). We conclude that STH's and *S. stercoralis* are highly prevalent in active-duty military personnel serving in tropical areas and massive drug administration may be warranted in this high risk population. Furthermore, clinicians should consider the worldwide distribution of *S. stercoralis* before starting any immunosuppressive therapy in war veterans returning from endemic areas.

753

PREVALENCE OF *BLASTOCYSTIS SPP.* AND *STRONGYLOIDES STERCORALIS* AMONG MILITARY SOLDIERS FROM PERUVIAN RAINFOREST

Marilhia Cornejo[1], Maria S. Gallardo[1], Diego Montoya[1], Jorge Urquiaga[1], George Vasquez[1], Renato Errea[1], Rodrigo Zamudio[1], Marco Canales[2], Angelica Terashima[2], **Jorge D. Machicado**[3], Luis A. Marcos[4]

[1]Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Instituto de Medicina Tropical Alexander von Humboldt, Lima, Peru, [3]University of Texas Health Science Center, Houston, TX, United States, [4]Forrest General Hospital, Hattiesburg, MS, United States

Blastocystis spp. is distributed worldwide and is the most frequent protozoa in human stools but is not commonly reported in field studies for its historically believed non-pathogenic role. A recent association between *Blastocystis spp.* and *Strongyloides stercoralis* has been suggested in a previous study from Japan. Despite school-aged children, others such as the military personnel on duty are also frequently affected by intestinal parasites. The aim of this study was to describe *Blastocystis spp.* and to determine its possible association with *Strongyloides* in military personnel of a local army of the Peruvian rainforest. A descriptive observational study was carried out in December 2012. One fresh stool sample from each participant was collected and evaluated by the direct smear, spontaneous sedimentation in tube technique and modified Baermann's technique. Data was analyzed by logistic regression to determine whether *Blastocystis spp.* was associated with *S. stercoralis* independently of other parasites. Among 104 individuals (age range: 15-34 years), the prevalence rate for *Blastocystis* spp was 54.8% (n=57) whereas the prevalence of *Strongyloides* was 28.8% (n=30). Two groups were analyzed separately, those with *Blastocystis spp.* versus those without this parasite in stools. The prevalence *of S. stercoralis* was higher in the group with *Blastocystis spp.* (70% vs. 30%) ($X2=3.92$, $p=0.04$). Logistic regression analysis confirmed the association between *Strongyloides stercoralis* and *Blastocystis spp.* (OR=3.1; CI=1.1-8.8; $p=0.03$), independently of the other parasites. We found an association between *Blastocystis spp.* and *S. stercoralis* in this military population, but the clinical importance and the underlying mechanisms of this association are unclear. These two parasites are highly endemic even in a non-school-aged population which constitutes another example of a neglected parasitic disease in the Amazonic region of Peru and further therapeutic interventions are needed.

754

ASSESSMENT OF THE EFFICACY OF ALBENDAZOLE FOR THE TREATMENT OF SOIL TRANSMITTED HELMINTHS IN EAST TIMOR

Susana V. Nery[1], Santina Gomes[2], Rebecca Traub[1], James McCarthy[3], Stacey Llewellyn[3], Ross Andrews[4], Darren Gray[1], Archie Clements[1]

[1]The University of Queensland, Brisbane, Australia, [2]National Health Laboratory, Dili, Timor-Leste, [3]Queensland Institute of Medical Research, Brisbane, Australia, [4]Menzies School of Health Research, Brisbane, Australia

Soil-transmitted helminths (STH), are among the most prevalent infections worldwide, and contribute to malnutrition and iron-deficiency anaemia, and adversely affect physical childhood growth. There has been a renewed global commitment to implement control strategies to reduce disease burden caused by STHs, particularly using regular periodic mass chemotherapy (MDA) with broad spectrum drugs such as Albendazole (ALB). The scale up of MDA will lead to increasing drug pressure on parasite populations, favoring the selection of drug-resistant parasites. In addition, the long term Public Health benefit of such MDA programs has been debated. The aim of this study, undertaken as part of randomised controlled trial looking at the impact of water, sanitation and hygiene (WASH) and ALB on STH infection, is to assess the antihelminthic efficacy of a single dose of ALB in communities in East Timor, where STH are endemic, by determining Faecal Egg Count Reduction (FECR) and Cure Rate (CR). Eligible community members (residents over one year of age and pregnant women in the first trimester) from 8 villages in Manufahi district were recruited. A total of 441 samples were collected immediately prior ALB distribution and 340 samples 7-12 days afterwards. All faecal samples were processed using a flotation technique for the detection and quantification of infections with STHs and protozoa. In addition, infection intensity was measured by multiplex qPCR. At baseline almost half of the participants were positive for *Ascaris lumbricoides* (44.8%), and hookworm (45.1%), while prevalence of T. trichiura was 1.1%. Very high cure rates were observed for *Ascaris*, while it was less efficacious against hookworms. While a single dose of ALB show high cure rates against STHs in Timor Leste, it will be important to monitor its efficacy over time, as the planned national MDA campaign is implemented. Furthermore, these results will have implications on the impact of the WASH intervention following ALB distribution on STH infection status; that is currently being trialed in the same area.

755

EPIDEMIOLOGY OF SOIL-TRANSMITTED HELMINTHS INFECTIONS AMONG ABORIGINAL SCHOOLCHILDREN IN RURAL MALAYSIA

Ahmed K. Al-Delaimy, Hesham M. Al-Mekhlafi, Nabil A. Nasr, Yvonne A. Lim, Rohela Mahmud

University of Malaya, Kuala Lumpur, Malaysia

Despite the continuous efforts to improve the quality of life of Orang Asli (Aborigines) communities, the prevalence of soil-transmitted helminth (STH) infections among these populations remains largely unchanged since the 1920s, with alarming high prevalence rates and prominent morbidity. This study aimed at investigating the current prevalence and potential risk factors of STH infections among Aboriginal primary schoolchildren in the Lipis district of Pahang state, Malaysia. Faecal samples were collected from 468 children (52.1% males and 47.9% females) and examined by using formalin-ether sedimentation, Kato Katz and Harada Mori techniques. Demographic, socioeconomic, environmental and behavioural information were collected by using a pre-tested questionnaire. Overall, 96.6% of the children were found to be infected by at least one STH species. The prevalence of trichuriasis, ascariasis and hookworm infections were 95.7%, 45.9% and 24.6%, respectively. Almost two-thirds and half of the trichuriasis, and ascariasis, respectively, were of moderate-to-heavy intensities while all hookworm infections were of light intensity. Univariate and multivariate analyses showed that absence of a toilet in the house, using unsafe water supply as a source for drinking water, presence of other family member infected with STH, inadequate knowledge on STH, not washing hands before eating, and not washing hands after defecation were the significant risk factors of STH infections among these children. In conclusion, there is an urgent need to implement school-based deworming programmes and other control measures like providing a proper sanitation, as well as a treated drinking water supply and proper health education regarding good personal hygiene practices. Such integrated control programme will help significantly in reducing the prevalence and intensity of STH in Aboriginal communities.

756

LONG TERM EXPERIENCE WITH STRONGYLOIDOSIS IN SINGLE CENTER IN NORTHERN ITALY

Giovanni Jacopo Nicoletti[1], **Enrico Brunetti**[1], Carmine Tinelli[2], Roberta Maserati[2], Antonella Bruno[2], Simona Gatti[2], Massimo Scaglia[1], Claudia Cevini[2], Francesca Tamarozzi[1], Stefano Novati[2]

[1]University of Pavia, Pavia, Italy, [2]Policlinico San Matteo, Pavia, Italy

We conducted a retrospective study at the Infectious Diseases Clinic of the University Hospital IRCCS San Matteo (Pavia, Italy), aimed at the identification of strongyloidiasis cases diagnosed between January 1983 and December 2011. This led to the identification of 1013 patients, of whom we conducted an analysis of demographic, clinical and laboratory features, and response to treatment on a sub-cohort of 536, seen from 1998 to 2011. the prevalence of missing data was considered statistically acceptable (<20%). These patients were mostly elderly (mean age 68 years), males (60.3%) and autochthonous cases (92.2%). In most cases direct exposure to soil, the most likely risk factor, occurred during domestic activities. Diagnosis was based on identification of larvae in stool samples or positivity of immunological tests (2 ELISA commercial kits). At least one significant comorbidity was present in 55.6% of patients and 18% were receiving immunosuppressant therapy with steroids. Nonetheless, only 10 patients (1.9%) showed laboratory features suggestive of severe strongyloidiasis. Early recognition and treatment of the infection could account for this severe strongyloidiasis. Early recognition and treatment of the infection could account for this finding, but genetic variation may also be a rason. Marked eosinophilia (>9%) was present in 80% of cases at the time of diagnosis. Gastrointestinal symptoms and pruritus were the most frequent complaints, followed by cutaneous lesions and respiratory signs. We found a strong association between respiratory symptoms and comorbidity, as well as corticosteroid therapy ($p<0.001$), and gastrointestinal symptoms and comorbidity ($p=0.024$). Treatment with ivermectin appeared more effective than albendazole, considering the lower rates of positivity after therapy. Although the majority of cases could derive from remote exposure, the high incidence of new diagnosis and the few but unequivocal cases of reinfection suggest that screening at-risk population for stronyloidiasis should not be discontinued and a careful follow-up should be carried out.

757

COMPARISON OF KATO-KATZ AND MINI-FLOTAC FOR THE DIAGNOSIS OF SOIL-TRANSMITTED HELMINTHS: RESULTS FROM A FIELD STUDY IN THE REPUBLIC OF CONGO

Sébastien D. Pion[1], Jean Bopda[2], Cédric B. Chesnais[3], Frédéric Louya[4], Andrew C. Majewski[5], Peter U. Fischer[5], Gary J. Weil[5], Michel Boussinesq[3], François Missamou[4]

[1]Institut de Recherche pour le Développement, Yaoundé, Cameroon, [2]Filariasis and Other Tropical Diseases Research Centre, Yaoundé, Cameroon, [3]Institut de Recherche pour le Développement, Montpellier, France, [4]Programme National de Lutte contre l'Onchocercose, Ministère de la Santé et de la Population, Brazzaville, Republic of the Congo, [5]Infectious Diseases Division, Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO, United States

Soil-transmitted helminths (STH) are ubiquitous in the developing world where they affect the poorest and most deprived communities. The World Health Organization recommends the Kato-Katz (KK) method for detecting STH infections in the field. Disadvantages of KK are that it must be performed on fresh stool samples, and KK slides must be read promptly to avoid overclearing of hookworm eggs. We compared the sensitivity of KK with that of Mini-FLOTAC, a newly developed field method for STH diagnosis that can be used with either fresh or preserved stool samples. Three methods were used to analyse stool samples from 284 Congolese individuals aged 5 years and over. Two Kato-Katz smears were prepared for each individual. In addition, one gram of fresh material was used for the Mini-FLOTAC (Fresh-Flo); another gram of stool was preserved in 5% formalin solution and tested by Mini- FLOTAC (Pres-Flo) three months later. A*scaris lumbricoides* was detected in 54.6%, 53.9% and 50.0% of samples using KK, Fresh-Flo and Pres-Flo techniques, respectively, with intensities of infection (geometric mean of positive counts) of 5558, 2778 and 2543 eggs per gram (epg). *Trichuris trichiura* was detected in 79.2%, 69.7% and 70.1% of samples using KK, Fresh-Flo and Pres-Flo, respectively, with intensities of infection of 373, 179 and 146 epg. Hookworm was detected in 6.7%, 19.7% and 4.2% of samples using KK, Fresh-Flo and Pres-Flo, respectively, with intensities of infection of 41, 53, and 19 epg. Mini-FLOTAC performed with either fresh or preserved samples was not superior to KK for detection and intensity assessment of *A. lumbricoides* or *T. trichiura* infections. However, Mini- FLOTAC appears to be more sensitive than KK for detecting hookworm eggs in fresh stool samples than the other methods. This advantage was lost after samples were stored for 3 months. Pres-Flo has the potential to release stool testing from the time constraints of KK. Additional studies are needed to determine how long preserved stool samples can be stored before hookworm eggs are lost.

758

EFFICACY OF SINGLE-DOSE ALBENDAZOLE FOR SOIL-TRANSMITTED HELMINTH INFECTIONS IN PERUVIAN SCHOOLCHILDREN

Theresa W. Gyorkos[1], Mathieu Maheu-Giroux[2], Brittany Blouin[1], Martin Casapia[3]

[1]The Research Institute of the McGill University Health Centre, Montreal, QC, Canada, [2]Harvard School of Public Health, Boston, MA, United States, [3]Asociación Civil Selva Amazónica, Iquitos, Peru

Soil-transmitted helminth (STH) infections contribute to the most disability-adjusted life years of all the Neglected Tropical Diseases, especially in school-age children, preschool-age children and women of reproductive age. To combat these infections, the World Health Organization recommends school-based, mass deworming programs in endemic areas along with continuous monitoring of drug efficacy in the community. The objective of the present study was to monitor the efficacy of single-dose albendazole for STH infection within the conduct of a school-based cluster randomized controlled trial in Peru. The study was conducted in Belén, in the state of Loreto, between April 21 and July 1, 2010. At baseline, stool specimens were collected from Grade 5 schoolchildren in 18 schools and analysed for STH prevalence and intensity using the Kato-Katz method. Following baseline assessment, all children were dewormed with single-dose albendazole (400 mg). Infected children were followed-up in schools two weeks following deworming and a second stool specimen was collected and analysed. A total of 385 children, infected with at least one STH species, participated in this follow-up study. The efficacy of albendazole was high for *Ascaris* (Egg Reduction Rate (ERR) = 98.8%; 95% confidence interval (CI): 92.1%, 99.9%) and hookworm infections (ERR = 86.3%; 95% CI: 71.6%, 93.0%) but much lower for *Trichuris* infection (ERR = 42.2%; 95% CI: 25.6%, 53.5%). These results are consistent with previous data published on the efficacy of albendazole. Efficacious preventive chemotherapy for *Trichuris* infections continues to be a challenge. Innovative research, including a back-to-biology-basics approach, to fully understanding *Trichuris* microepidemiology, may provide additional insight.

759

THE *NA*-GST-1 HOOKWORM VACCINE IS SAFE AND INDUCES NEUTRALIZING ANTIBODIES IN BRAZILIAN ADULTS

David Diemert[1], Vanderson Valente[2], Janaina Freire[2], Shannon Grahek[1], Maria V. Periago[2], Maria F. Gazzinelli[3], Marconi Fernandes[3], Cassia Senra[2], Renata Diniz[2], Rodrigo Correa-Oliveira[2], Peter J. Hotez[4], Jeffrey Bethony[1]

[1]George Washington University, Washington, DC, United States, [2]Centro de Pesquisas Rene Rachou, Belo Horizonte, Brazil, [3]Federal University of Minas Gerais, Belo Horizonte, Brazil, [4]National School of Tropical Medicine at Baylor College of Medicine, Houston, TX, United States

Necator americanus glutathione S-transferase-1 (*Na*-GST-1) is a 24-kDa protein that catalyzes the conjugation of reduced glutathione to a variety of electrophiles. *Na*-GST-1 belongs to a class of nematode GSTs characterized by diminished peroxidase activity relative to other GSTs but increased binding capacity for heme and related products. It is produced by adult hookworms and is thought to play a role in detoxifying heme and other breakdown products of the hookworm blood digestion pathway. Vaccination of laboratory dogs and hamsters with recombinant GST-1 results in reduced hookworm fecal egg counts and adult worm burden following challenge with infective larvae. Recombinant *Na*-GST-1 was expressed in *Pichia pastoris* and formulated with Alhydrogel. 36 healthy Brazilian adults with no history of hookworm exposure and living in the city of Belo Horizonte were enrolled in a dose escalation Phase 1 clinical trial of *Na*-GST-1. Volunteers received 1 of 3 different dose concentrations of *Na*-GST-1 (10, 30 or 100 µg) in 1 of 2 different formulations (*Na*-GST-1/Alhydrogel or *Na*-GST-1/Alhydrogel to which 2.5 µg of the Toll-like receptor-4 agonist, glucopyranosyl lipid A [GLA-AF], was added as a point-of-injection preparation). Subjects received 3 intramuscular injections at 2-month intervals. Subjects were screened by ELISA for serum IgE antibodies to *Na*-GST-1 due to the previous experience with IgE-related urticarial reactions induced by the recombinant *Na*-ASP-2 hookworm vaccine; all were negative. Common vaccine-related solicited adverse events included mild to moderate injection site pain and tenderness (2 cases of severe pain), headache (1 severe), and nausea; no differences between dose groups were observed in incidence of adverse events. Anti-*Na*-GST-1 IgG antibody levels as measured by ELISA were modest after the 2nd vaccination, but increased significantly from baseline after the 3rd vaccination in those who received 100 µg *Na*-GST-1. For each dose concentration of *Na*-GST-1, the increase in IgG levels was not significantly different in those who received formulations containing GLA-AF. Importantly, induced IgG antibodies inhibited binding of heme by *Na*-GST-1 in an *in vitro* assay. These data demonstrate that *Na*-GST-1/Alhydrogel is well tolerated and induces anti-*Na*-GST-1 IgG antibodies that have functional activity in inhibiting the target antigen. Further testing in hookworm endemic populations is on going.

760

IMPACT OF PERIODIC SELECTIVE MEBENDAZOLE TREATMENT ON SOIL-TRANSMITTED HELMINTH INFECTIONS IN CUBAN SCHOOLCHILDREN

Suzanne van der werff[1], Kim Vereecken[2], Kim van der Laan[1], Maiza Campos Ponce[1], Raquel Nunoz[3], Fidel A. Nunez[4], Lázara Rojas Rivero[4], Mariano Bonet Gorbea[3], **Katja Polman**[2]

[1]VU University, Amsterdam, Netherlands, [2]Institute of Tropical Medicine, Antwerp, Belgium, [3]National Institute of Hygiene, Epidemiology and Microbiology, Havana, Cuba, [4]Pedro Kourí Institute, Havana, Cuba

Periodic treatment with 500 mg mebendazole is one of the strategies currently recommended by the WHO to control soil-transmitted helminth (STH) infections in endemic areas. The efficacy of anthelminthic drugs has been investigated within randomized controlled trials (RCTs). Various studies exist on the effectiveness of periodic anthelminthic treatment, but mainly in the context of (targeted) mass treatment studies. Here, we evaluated the impact of periodic selective treatment with mebendazole on STH infections in Cuban schoolchildren. We followed up a cohort of 268 STH-positive schoolchildren -aged 5-14 years at baseline- at six months intervals for two years and a final follow-up after three years. The Kato-Katz stool examination technique was used to detect infections with *Ascaris lumbricoides*, *Trichura trichiura*, and hookworm. Common risk factors related to STHs were assessed by parental questionnaire. A significant reduction in the number of STH infections was obtained after three years with the highest reduction for *T. trichiura* (87.8%) and the lowest for hookworm (57.9%). After six months cure rates (CRs) were 76.9% for *A. lumbricoides*, 67.4% for *T. trichiura*, and 44.4% for hookworm. After two treatment rounds, more than 75% of all STH positive children at baseline were cured, but with important differences between STH species (95.2% for *A. lumbricoides*, 80.5% for *T. trichiura*, and 76.5% for hookworm). At the end of the study, these cumulative CRs were almost 100% for all three STHs. Risk factors for STHs were sex, sanitary disposal, and habit of playing in the soil. Our results indicate that periodic selective treatment with a single dose of 500 mg mebendazole is effective in reducing the number of STH infections in Cuban schoolchildren. Although important differences were found between helminth species, two rounds of treatment appeared sufficient to obtain substantial reductions.

761

THE IMMUNOLOGICAL RESPONSE OF HIV POSITIVE PATIENTS INITIATING HAART AT THE KOMFO ANOKYE TEACHING HOSPITAL, KUMASI, GHANA

Lawrence Annison[1], Albert Dompreh[2], Yaw Adu-Sarkodie[3]

[1]Kumasi Centre for Collaborative Research in Tropical Medicine, Kumasi, Ghana, [2]Serology Unit, Komfo Anokye Teaching Hospital, Kumasi, Ghana, [3]Department of Clinical Microbiology, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Highly active antiretroviral therapy (HAART) is the mainstay of treatment for people living with HIV (PLHIV). Although there is enough documented evidence on immunological response of patients to HAART elsewhere, in Ghana, it is not well documented. We document the experience of immunological improvement among Ghanaian PLHIVs on HAART, comparing different categories of patients. Questionnaires were used for patient demographic and clinical data. Four CD4 counts were measured at six-monthly intervals to determine and compare rates of CD4 change among different categories of patients. Women had higher CD4 count (77.4cells/µl) at baseline. CD4 count increased from a mean baseline of 70.2 cells/µl to 229.2, 270.0, and 297.6 cells/µl at 6, 12, and 18 months of treatment respectively ($p < 0.0001$). There were no gender ($p=0.46$) and age ($p=0.96$) differences in treatment response. There was no difference ($p=0.18$) in treatment response comparing patients with CD4 <250 cells/µl and those with CD4 count between 250-350 cells/µl. Out of 282 patients with pre-therapy CD4 count ≤250 cells/µl, 241 (85.5%) and 41 (14.5%) were adherents and non-adherents respectively. Mean rate of increase was 15.2 and 8.4 cells/µl/month in adherents and non-adherents respectively ($p=0.2$). The findings of this study suggests that a sustained CD4 increase could be achieved in adherent patients commencing therapy with baseline CD4 count <250 cells/µl. These patients have greater ability for immunological recovery during 12 months of treatment. We, therefore, conclude that significant immunological improvement is therefore possible among Ghanaian PLHIV on HAART as long as a high level of adherence is observed.

762

HIV COUNSELING AND TESTING AMONG PATIENTS WITH TUBERCULOSIS AT ARBAMINCH HOSPITAL, SOUTHERN ETHIOPIA

Rediet Abebe, Mistire Fantahun, Yirgalem Hailemicheal, Mekides Yizengaw, **Mulugeta M. Zegeye**

Arbaminch University, Arbaminch, Ethiopia

Ethiopian National tuberculosis (TB) and Human Immune Virus (HIV) guideline that recommend HIV counseling and testing as part of routine TB care was in effect since 2005. However, the number of TB patients that know their HIV status remains low in the country. The objective of our study was to assess the HIV counseling and testing among TB patients at Arbaminch Hospital, Southern Ethiopia. We conducted a cross sectional study from January to April 2012 at Arbaminch Hospital (AMH). Newly diagnosed TB patients who fulfilled the inclusion criteria were enrolled for this study. The sample size was calculated using a single proportion formula and participants were recruited sequentially. Socio-demographic and TB/HIV related information of study participants were collected using pre-tested interviewer administered questionnaire. The HIV status and other clinical data of study participants were taken from the TB treatment registration book in the TB clinic. We enrolled a total of 76 newly diagnosed TB patients. The majority of study participants (92.1%) reported that they have been consulted by physician to take an HIV test when they were diagnosed with TB. Among study participants consulted by physician to get HIV testing, 54.3% did not receive counseling service. One-fourth of patients who received the counseling service did not go on to get tested. Overall, 23.7% of the study participants were receiving anti-TB treatments without their HIV status determined. None of patient related factors we assessed were associated with obtaining consultation and counseling services and with willingness to get tested except for residence ($p=0.041$) and previous HIV screening history ($p=0.004$). In conclusion, the HIV counseling and testing service given to TB patients in the Hospital was low and poorly harmonized which calls for alternative strategies to improve willingness of TB patients to be tested; and meliorating awareness of physicians on the benefits of the testing. It also sounds like improving the coordination between physicians and counselors is vital if providing consultation, counseling and testing services in one setting is not possible.

763

THE EFFECTS OF MALARIA AND HIV CO-INFECTION ON HEMOGLOBIN LEVELS IN PREGNANT WOMEN IN SEKONDI-TAKORADI METROPOLIS, GHANA

Verner N. Orish

Effia Nkwanta Regional Hospital Sekondi Western Region, Sekondi, Ghana

This study was undertaken to assess the burden of malaria and human immunodeficiency virus (HIV) co-infection and to determine the risk of anemia among dually infected pregnant women in Sekondi-Takoradi, Ghana. A cross-sectional study was conducted at four hospitals in the Sekondi-Takoradi metropolis comprising 872 consenting pregnant women attending their antenatal care clinics, cross-checked with ultra sound or with clinical evidence of pregnancy. The study showed that 34.4% of the pregnant women had anemia while 65.6% were non-anemic. Multivariable logistic regression analysis indicated that pregnant women with a single infection with either malaria or HIV were independently associated with increased odds of maternal anemia. In adjusted models, pregnant women co-infected with malaria and HIV doubled their risk of maternal anemia (adjusted OR, 2.67, 95% CI, 1.44-4.97, P = 0.002). In conclusion, dually infected pregnant women with malaria and HIV are twice likely to be anemic than those with a single or no infection. For all pregnant women in this region, it is imperative to control for malaria, HIV and anemia in other to improve birth outcomes.

764

MOLECULAR TYPING OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM RESPIRATORY TRACT OF HIV-POSITIVE CHILDREN AND ADOLESCENTS IN CAMBODIA

Nada Kulkova[1], Jaroslava Sokolova[2], Anna Liskova[1], Andrea Shahum[3], Vladimir Krcmery[1]

[1]St. Elizabeth University, Bratislava, Slovakia, [2]Trnava University, Department of Laboratory Medicine, Trnava, Slovakia, [3]St. Maximilian Kolbe Clinic, Phnom Penh, Sihanoukwile, Cambodia

HIV-infection is an important risk factor for acquisition of methicillin-resistant *Staphylococcus aureus* (MRSA)-associated infection. Molecular typing methods are useful for study of relationships of outbreak-causing strains in health-care settings, but many of them are time-consuming, expensive and require experienced stuff. In this study we aimed to assess incidence of MRSA in group of HIV-positive Cambodian children and adolescents and to evaluate applicability of HVR-mecA-typing for detection of clonal MRSA spread. Samples from HIV-positive patients (n=42) living in two long-term health care facilities for orphans in Cambodia (Phnom Penh and Sihanoukwille, respectively) were included. Bacteria retrieved from respiratory swabs in 2006 and 2012 were analyzed. Altogether, 156 isolates were obtained, of which 56 (35,9 %) were identified as S. aureus and 29 (18,6 %) of these were MRSA. Eighteen (n=18) MRSA isolates recovered from stock cultures were included for further typing of hypervariable region of mecA gene (HVR-mecA-typing) to assess polymorphisms in direct repetitive units (DRU). HVR-mecA-typing revealed six different HVR-types, with HVR-type I (fragment length 575 bp) being the most prevalent (41,2 %), followed by types II and V (17,5 % both). Considering resistance, except of methicillin resistance, erytromycin resistance was the most frequent (88,9%), followed by clindamycin (61,1%) and cefuroxim (61,1 %) resistance. No correlation between

resistance pattern and HVR-type was observed. Interestingly, respiratory tract infections, but not skin and soft tissues infections, were the most common in patients with MRSA. According to typing results we assumed evolution out of one or two different SCCmec resources. As the HVR-types are a lot heterogenous, we suppose that the spread of MRSA in those particular facilities is not clonal, but the HVR has diverged overtime. This is interesting, considering fact that in health care settings MRSA are usually spread clonally.

765

FIRST INDIRECT MEASURE OF INCIDENCE AND RISK FACTORS FOR RECENT INFECTIONS WITH HIV-1 AMONG FEMALE SEX WORKERS IN THE DISTRICT OF BAMAKO, MALI

Modibo Keita[1], Seydou Coulibaly[1], Yaya Ibrahim Coulibaly[2], Ilo Dicko[2], Sanata Sogoba[3], Fatoumata Binta Traore[1], Issiaka Dembele[4], Mamoudou Kodio[1], Boubacar Diallo[1], Oumar Maiga[1], Samba O. Sow[1], Somita Keita[1], Adama Faye[5]

[1]National Support Center for the Fight of Disease/Center of Vaccine Development, Bamako, Mali, [2]Filariasis Unit, Malaria Research and Training Center, Bamako, Mali, [3]National Assessment Agency of Hospitals, Bamako, Mali, [4]Ambulatory Unity of Cares and Advice about HIV/AIDS, Bamako, Mali, [5]Cheick Anta DIOP University, Dakar, Senegal, Bamako, Mali

In Mali, HIV is a public health problem with an overall prevalence of 1.3% in the general population. This prevalence reaches 26% in some risk groups such as female sex workers. The incidence of HIV, an important element in assessing the impact of control interventions, has never been evaluated in Mali. The recent settling of HIV incidence tests and the UNAIDS "vision three zero" have paved the way for indirect measures of HIV incidence among vulnerable populations. We conducted a cross-sectional survey involving 388 sex workers in Bamako who underwent interview, diagnostic tests (HIV-1/2) and incidence tests of HIV-1 with the BED-EIA (Enzyme Immuno Assay) to identify recent infections in women infected with HIV. The socio demographic characteristics, the factors related to health services and those related to the practice of sex work were taken into account in a multinomial logistic regression model to assess the associated risk for a recent infection as compared to the non-infected. Among the 388 sex workers included with a median age of 25 years (18-53), 71 (18.3%) were seropositive to HIV-1. Based on the adjustment method of Kassanjee *et al*, the incidence was high with [8.84%; 95% CI (4.21 to 13.46)]. The age 35 and more , the number of years worked as a sex worker, the number of customers per day greater than 5 were the main risk factors for recent HIV-1 infection with respectively [OR = 5.94 (1.03 to 34.15)], [OR = 1.18 (1.01 to 1.39)] and [OR = 17.87 (1.87 to 208.92)] at 95% CI. The participation to a VIH screening test less than a year ago, the interaction between education status and the number of customers per day greater than 5 were protective factors against a new HIV infection with respectively [OR = 0.25 (0.07 - 0.87)] and [OR = 0.03 (0 - 0.41)] at 95% CI. The overall incidence of HIV-1 infection was high among sex workers in the district of Bamako in 2012. The development of professional reorientation programs of sex workers in the context of improving the HIV prevention activities in certain risk groups may help reduce this incidence.

766

THE DISTRIBUTION PATTERN OF SEXUALLY TRANSMITTED INFECTIONS AMONG FEMALE CLIENTS VISITING FOR MEDICAL LABORATORY TESTS IN LAGOS, NIGERIA

Jones U. Nwadike

Dunamis Diagnostic and Research Center, Lagos, Nigeria

Eight hundred and seventy-nine female clients reporting for medical Laboratory diagnosis were screened in a medical diagnostic laboratory in Lagos, Nigeria. Blood sample was collected from 246 (28.0%) clients for serological screenings, while urine and High vagina swab (H/V/S) samples were collected from 633 (72.0%) for microbiological analysis and culture. The women were of child bearing age with range between 15 - 42 years old. 332(37.8%) of the clients were not married while 547(62.2%) were married women. The mean age of the un-married women was 26.7 (±3.25 SD) while married was 33.80 years (±10.35 SD). There was no difference in ages (p-value = 0.4). The group age of the participants include 15 - 20; 21- 25; 26 - 30; 31- 35; 36- 40 and 41+. Overall, urinary tract infection (UTI) caused by bacteriological organisms was top in the list of infections 357/879(40.6%) + 16(1.8%) followed by HIV types 1 and 11 91/879 (10.4%); Candidiasis 48/879(5.5%); while Syphilis, genital warts and hepatitis B. Infection were 2(0.2%) each. Married women were more infected with UTI 214/357(60.0%) compared to unmarried 143/357(40.0%), infection was statistically significant with p-value =0.1. Similarly more married women were more infected with HIV 54/91(59.3%) compared to unmarried 37/91(40.7%). Infection varies and increases with age, among the married UTI was more with the 26-30 age group 90/214(42.0%) while among the unmarried it was more with the 21- 25 age group 67/143 (46.8%). HIV infection was more with the 31- 35 group age among the married 32/54(59.2%) while among the unmarried it was more with the 21- 25 age group 16/37 (43.2%). More married women were infected with candidiasis 32/48(66.7%) with the 26-30 age group 9/32(28.1%) topping the others compared to unmarried 16/48(33.3%) with more infection among the 21- 25 age group 10/16(62.5%). High prevalence rate of sexually transmitted infections is a public health risk. Clinicians and program managers should promote routine screening of all pregnant women for infections and early treatment for both married and unmarried population while providing community health education to reduce spread.

767

BARRIERS TO UTILIZATION OF PROVIDER-INITIATED HIV COUNSELING AND TESTING SERVICES AMONG TUBERCULOSIS PATIENTS: A CASE OF RHODES CHEST CLINIC NAIROBI, KENYA

Joab Khasewa

National AIDS Control Council, Kenya, Nairobi, Kenya

Tuberculosis (TB) continues to be one of the most important global public health threats. HIV prevalence among TB patients in Sub-Saharan Africa is 70%.In Kenya, over 60% of TB patients are HIV positive(MOH,2007). The objective of the study was to determine the barriers to utilization of provider initiated HIV counseling and testing services among TB patients. A cross sectional survey of TB suspects visiting chest clinic was conducted. Consenting patients who visited the clinics during October to December 2010 were the study subjects. Data was collected through structured interviews with TB patients visiting the facility using a standard questionnaire and direct observation. The quantitative data was analyzed using descriptive statistics. A chi square test was used to interpret results for each possible barrier in terms of utilized versus declined to utilize HIV counseling and testing services. The test was considered to be statistically significant if the P-value was < 0.05. It was found that 83 % of TB patients tested for HIV infection. The main reasons for not being tested were that they don't trust confidentiality (17.9%), fear of positive test results (11.9%), fear of discrimination (10.4%), fear of being stigmatized (9.0%) and self perception of low risk (7.5). (χ2=29.473, 9 df, p=0.030). Factors that were significantly associated with utilization of PITC services were level of education (χ2=116.045,2df,p=0.0001), HIV stigma (χ2=36.947,3df,p=0.0001), awareness of HIV-TB link (χ2=22.767,2df,p=0.0001) and discussion of HIV/ TB link by nurse (χ2=59.232,2df,p=0.0001). In conclusion, TB patients evidently experienced both patient related and provider based barriers.The NACC, NLTP and TB/HIV Partners should scale up community awareness about HIV-TB co infection and train all providers on collaborative HIV-TB services. Advocacy for HIV screening for all TB patients should also be increased.

768

ADVERSE NEUROLOGICAL EVENTS DUE TO ANTIRETROVIRAL THERAPY IN MALI

Naguibou M. Djibril

UAC (Benin State University), Cotonou, Benin

Adverse neurological events during antiretroviral treatment (ART) are frequent and various. Their diagnosis occur some difficulties in different orders according to the geographical context. To identify the frequency of neurological side effect, we performed prospective study taking care of any patient under antiretroviral treatment and developing neurological manifestations in a period of 12 month in infectious diseases service located at the teaching hospital "Point G", Bamako, Mali. Neurological diagnoses have been done with the guidance of a neurologist. All data were collected in a file. Side effects classification according to WHO has been used to characterize them[4]. Analysis of data has been done in the Software SPSS version 12.0. Four hundred and twenty two (420) HIV seropositive patients under ART treatment have been followed. Among them, 37 cases have been discovered with adverse neurological events (8.08%). The sex ratio M/F was 1.06. The age average was 41.2 years. Polyneuritis alone represented 83.8%, and then Polyneuritis associated to vertigo, headache and depression represented 16.2 %. We didn't notify any neurologic symptoms at the ART initiation. The major part was infected by HIV-1(91.9 %). 89.2% of them were under fixe dose combination Triomune® (D4T+3TC +Nevirapine). Five cases were at 3rd stage of WHO classification (13.5%) what justified consequently the stopping of d4T. Nevertheless, adverse neurological events may arise by using Triomune®. In future antiretroviral therapy must take into account neurological consequences and the instauration of pharmacovigilance to detect eventual drugs with neurological side effect.

769

RELATIVE LOW NUMBER OF NEW HIV CASES DETECTED IN RURAL DISTRICT BUNDA IN NORTHWEST TANZANIA

Gertruda Mikolasova[1], Ch. Bonnach[2], C. Tim Wo Monte[2], Renata Machalkova[2], Juraj Benca[2], Andrej Karkalik[2], Eva Vojtikevicova[2], Zuzana Kudlova[2], Jaroslava Sokolova[3], Nada Kulkova[4], Vladimir Krcmery[5]

[1]Slovak Tropical Institute, Bratislava, Slovakia, [2]St. Elizabeth University Tropical Program, Kibara Hospital, Bunda, United Republic of Tanzania, [3]Department of Laboratory Medicine, Trnava University, Trnava, Slovakia, [4]Laboratory of Molecular Microbiology, St. Elisabeth University, Bratislava, Slovakia, [5]St. Elizabeth University College of Health and Social Care, Bratislava, Slovakia

HIV prevalence in East Africa is still of concerns and was initially 15% - 25 % in Uganda, Kenya and Tanzania in 1995 - 2005. However, it decreased gradually to 10% - 20% after introduction of ABC educational campaign, point-of-care testing during outbreaks in 2010, mother-to-child-transmission prevention (MTCTP) since 2003 and increasing number of treated patients and after 2005 global Fund and world Bank introduced free generic antiretroviral payment (e.g. NASCOP) in Kenya. The aim of this study was to determine HIV occurrence in newly tested individuals in distinct hospital voluntary counselling and testing (VCT) and AIDS cases in Bunda, Tanzania. Altogether 14499 patients came at study period to outpatient department (OPD) and inpatient (IPD) units (general, internal unit, paediatric, surgical, and maternity units). These were treated since July 2011 to August 2012, with an average of 1200 - 1300 patients a month (674 - 789 OPD and 363 - 514 IPD). Incidence of new HIV-positive cases, malaria, tuberculosis cases, pneumonia and diarrhoea as well as sexually transmitted diseases (STD) were monthly assessed. Within last 5 years, 1194 new cases of HIV were detected (in 2005 - 2010) and 226 (19%) of these patients receive antiretroviral therapy (ARV), which counts for approximately 20 new treated cases per month (240 a year). Of 1037 - 1210 monthly visits, about 400 - 500 were tested, which is 3 - 4% prevalence, even in sick patients population. Since July 2011, monthly prevalence was 10-15 cases/1000 -1200 patients (1 - 1,5%), which is much less than prevalence reported by government in West Tanzania. Low prevalence of HIV positive patients in Kibara Hospital is constantly decreasing from 8 - 15% as reported by WHO to 3 - 4% in 2005 - 2010 and even to 1,5-3% in 2011/2012. This is probably due to active outreaching and screening policy, access to VCT, free ARV, testing and ABC educational campaign in Tanzania.

770

ROLE OF CARE AND TREATMENT CENTERS ON INFLUENCING ADHERENCE TO ANTIRETROVIRAL THERAPY BY HIV PATIENTS IN DAR ES SALAAM

Fausta S. Mosha[1], Eliangiringa Kaale[2], David Ocheng[3], Peter Risha[4], Victor Muchunguzi[1], Raphael Z. Sangeda[5], Eligius Lyamuya[2], Anne-Mieke Vandamme[5]

[1]Ministry of Health and Social Welfare, Dar es Salaam, United Republic of Tanzania, [2]Muhimbili University of Health and Allied Sciences, Dar es Salaam, United Republic of Tanzania, [3]Health Links Initiative, Dar es Salaam, United Republic of Tanzania, [4]PharmAccess International, Dar es Salaam, United Republic of Tanzania, [5]Rega Institute for Medical Research, Department of Microbiology and Immunology, Leuven, Belgium

HIV care and treatment centres (CTC) are being established to support HIV patients to live a healthy life. It is important to investigate how CTC attendance relates to patient adherence to antiretroviral therapy (ART) and ultimately influence treatment response. For this reason, the patients' adherence to ART, and associated factors were correlated and the role of CTC in influencing this was evaluated. This was an analytical cross-sectional study conducted in Dar es Salaam. Data abstraction was done using structured questionnaires and records review. Descriptive analysis was done for the demographic and clinical characteristics. The chi-square and multivariate logistic regression analysis were used to identify factors related to adherence. Four hundred and twelve patients attending CTC in Dar-Es-Salaam were recruited. There were 134 (33%) males with median age of 42 years. Sixty five (16%) had post primary school education, 121 (29%) had no stable income and 98 (24%) had an income of more than 100 USD per month. Two hundred and nine (51%) were self-employed, 138 (33%) were unemployed. Three hundred and two (73%) have not been on care before starting ART and 316 (77%) had been on ART for more than 12 months. Two hundred and fifty two (61%) reported side effects while on ART and 233 (57%) perceived care at CTC as very good. The prevalence of adherence based on consistency of keeping appointments was 314 (76%), based on three days recall was 362 (88%) and based on taking ARV more than 90% of the time was 234 (57%). Our findings consistently found high adherence prevalence based on three days recall and consistency of keeping appointments. The key factors affecting adherence were; missing appointment and registering late to CTC. We found that provision of adequate education on the importance of strict adherence to the prescribed doses of ARVs during regular attendance to CTCs is an important factor towards achieving good adherence to ART. Future research should explore what factors in rural setting in Tanzania presents barriers to adherence.

771

HUMAN AFRICAN TRYPANOSOMIASIS UNCERTAINTY IN GHANA

Naa O. Ammah[1], Kwabena M. Bosompem[1], Charlse I. Mahama[2], John Larbi[3]

[1]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [2]Ghana Veterinary Services Department, Accra, Ghana, [3]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Some countries in the West African Region, including Burkina Faso and Cote d'Ivoire have reported cases of Human African Trypanosomiasis (HAT) in the past decade. Ghana, after the control of the HAT epidemic in the 1940's, has not had active surveillance for the disease. There have however

been sporadic reports of HAT in the last decade, especially, in the Western Region of Ghana. HAT is not seen to be of public health concern in Ghana thus not considered by doctors in major hospitals as cause of sickness in patients reporting to health centres although tsetse flies, the vectors of the disease, occur in many parts of the country. Symptoms of the disease are similar to diseases such as malaria and common flu, making the situation a serious one as when left untreated, patients become reservoirs. This study aims to identify species of trypanosomes in naturally infected flies, using pig farms in the Eastern Region, Ghana, as a case study. Pigs and other domesticated ruminants have been incriminated as reservoirs for *Trypanosoma brucei gambiense*, causative agent of HAT. The region has in past years recorded cases of trypanosomiasis in pigs with farmers incurring up to 50% losses within 2006/2007. The Eastern Region is located in the southern sector of Ghana and is mostly forest vegetation. *Glossina palpalis*, vector for the HAT, is the dominant tsetse species in that region. Biconical traps were set close to sties in five pig farms and trapped tsetse flies were morphologically identified and sorted then, non teneral flies were dissected to collect mid guts, hypo pharynx and salivary glands. Molecular analyses of the samples are on- going to determine the tsetse flies' sources of blood meal and the Trypanosoma species they may be infected with. This process is expected to be completed by mid- April. Results and data will be presented. Results from the study are expected to shed more light on the situation of HAT in Ghana by revealing the species of trypanosomes circulating in the system; examine the pattern of transmission and determine chances of an outbreak of HAT in within that area. This will inform the appropriate monitoring and surveillance methods to curb possible future epidemics.

772

THE HUMAN AFRICAN TRYPANOSOMIASIS (HAT) IN SENEGAL: MULTIDICIPLINARY APPROACH FOR AN IDENTIFICATION OF THE AREAS AT RISK

Massamba Syll[1], Daouda Ndiaye[1], Therese Dieng[1], Aly Gueye[1], Badara Cisse[1], Mamadou Ndiaye[1], Fabrice Courtin[2], Jean Louis Ndiaye[1], Babacar Faye[1], Omar Gaye[1], Oumar Ndir[1], Perez Simaro[3]

[1]UCAD, Dakar, Senegal, [2]CIRDES, Bobo Dioulasso, Burkina Faso, [3]World Health Organization, Geneva, Switzerland

Today the trypanosomiasis always represents public health problems in some countries of sub-Saharan Africa, even if it is recognized in a general way that the situation clearly improved following the example of West Africa. However the situation under area remains fuzzy since very little, even not of information are available for 6 countries, as Senegal which formerly sheltered active hearths of trypanosomiasis. Besides many areas of West Africa were not visited since independences. In Senegal the history of the HAT goes back to the colonial period. The first cases of trypanosomiasis were announced on the Small Coast by Sice which describes them under the name of "Nélawane". Many cases were observed of 1900 the day before independences, in the areas contaminated like the Delta of Senegal, Niayes, the Small Coast, Casamance and Eastern Senegal. The disease had almost disappeared in the Sixties, at which time she was then regarded as residual. The last case recorded in Senegal goes back to 1977 in a 18 year old young man born in Mbour an old active hearth. Since then, no case of disease of the sleep was announced, but also no program of epidemiologic monitoring was set up. Only a project of fight against the glossines in Niayes and part of small the east coast in the course of execution. However after having revisited the principal historical hearths of Senegal, we studied the distribution and the densities of glossines via the degree of development of hydraulic works as well as the hydrographic network and pluviometry in the areas formerly contaminated by the HAT. The analysis of these various factors besides the migratory bond with the infected countries made it possible to identify the areas at the risk of HAT namely: the Low one and High Casamance, the mouth of Sine Saloum, the zone of Kédougou and Niayes. Thus a reactualization of the epidemiologic and entomological data is essential.

773

EVALUATING THE INTERRUPTION OF *TRYPANOSOMA CRUZI* TRANSMISSION IN COMMUNITIES WITH REEMERGING VECTOR POPULATIONS

Jennifer Manne[1], Corentin M. Barbu[2], Ricardo Castillo Neyra[3], Michael Z. Levy[2]

[1]Harvard School of Public Health, Boston, MA, United States, [2]University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, United States, [3]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

In order to assess the effectiveness and achievements of vector control programs for Chagas disease, evaluation goals and accompanying procedural guidelines have been defined. However, as some programs struggle with the concurrent challenges of persistent reinfestation, instituting and maintaining robust vector surveillance programs and sustaining interest and funding for vector control activities, the need to reassess and strengthen evaluation procedures has become increasingly relevant. One particular concern in the context of evaluating Chagas disease control programs is how best to optimize the selection of indicators for evaluation and their application in the evaluation process. Drawing on data from the published literature and reports produced by national and international institutions, we offer a review of the evaluation process for Chagas disease vector control programs. First, we describe the principles and existing evaluation process for these programs. Next, we review the historical origins of the use of seroprevalence in children between 0 and 5 years old as a primary indicator for evaluation. We then examine the alternative indicators to assess progress toward interruption of transmission and conduct a critical review of the current recommended evaluation procedures. We identify seven key indicators that may be used to evaluate Chagas disease vector control programs. The origin and historical application of these indicators are further examined, along with the advantages and weaknesses associated with their use in the evaluation process. In addition, we offer strategies for strengthening the evaluation process by identifying several key areas for improvement in the current evaluation process, including the importance of using a complement of indicators to measure progress toward interruption of parasite transmission and the need for more stringent adherence to periodic surveillance after certification.

774

SCREENING OF POTENTIAL ANTI-*LEISHMANIA*L DRUGS AGAINST INTRACELLULAR *LEISHMANIA* BY A HIGH-THROUGHPUT DRUG SCREENING FORMAT

Debalina Ray, Jair L. Siqueira-Neto, Geraldine De Muylder, James Mckerrow

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

Leishmaniasis is caused by protozoan parasites of the genus *Leishmania*. The disease is highly endemic in tropical and sub tropical areas and the Mediterranean basin, threatening around 350 million people worldwide. The parasite is transmitted to the mammalian host by the bite of phlebotomine sand flies. There are three dominant forms of clinical leishmaniasis. The most common form is cutaneous leishmaniasis usually presenting as solitary ulcer. Muccocutaneous leishmaniasis can cause disfiguring skin lesions. The most severe form is visceral leishmaniasis, where the parasite migrates to liver, spleen and other organs. If untreated, death may occur. There is still no vaccine protection against leishmaniasis, so chemotherapy is critical. Only two drugs sodium stibogluconate and amphotericin B are in currently used widely. They have several side effects. There is, therefore a need for new cost-effective drugs for the treatment of leishmaniasis. Primary drug screening is often apprised out with the promastigote stage of parasite since it is easy to maintain in the laboratory. But an anti-leishmanial drug should be tested against the intracellular form or in amastigote. We have developed a host cell based drug screening assay using a human macrophage cell line infected with the causative

agent of visceral leishmaniasis, *Lesihmania donovani*. This assay format directly screens compounds against the intracellular form of *Leishmania*. We are currently collaborating with different institutes, companies and universities to screen thousands of compounds against the intracellular parasite. Results of these screens will be presented.

775

WHOLE BLOOD IFN-Γ RELEASE ASSAY IN IDENTIFYING EXPOSURE TO *LEISHMANIA DONOVANI* INFECTION IN HIGHLY ENDEMIC VILLAGES IN BIHAR, INDIA

Om Prakash Singh[1], Abhishek Kumar Singh[1], Neetu Singh[1], Kamlesh Gidwani[1], Ankita Chaurasia[1], Epco Hasker[2], Rudra Pratap Singh[1], Albert Picado[2], David Maclean[3], David Sacks[4], Marleen Boaeleart[2], Shyam Sundar[1]

[1]Institute of Medical Sciences, Banaras Hindu University, Varanasi, India, [2]Department of Public Health, Institute of Tropical Medicine, Antwerp, Belgium, [3]Cellestis Limited, Chadstone, Victoria, Australia, [4]Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

Of all persons infected with the parasites causing visceral leishmaniasis (VL) usually only 10-25% progress to clinical disease. We have recently developed a whole blood IFN-γ release assay (IGRA) as a marker of cellular immunity to detect infected and non- infected persons with high accuracy. In the present study, we employed this test along with quantitative PCR (qPCR) amongst healthy individuals living in the kala-azar endemic zone in Bihar, India for identification of individuals exposed to leishmania infection but without disease. We enrolled 13,163 persons from eleven highly endemic villages to identify the incident infected healthy persons as measured by seroconversion with DAT and rK39 ELISA at base line survey and at 12 months interval. Whole blood assay and qPCR were performed longitudinally for two years amongst seropositive and its matched seronegative controls populations. Level of IFN-γ was determined in antigen stimulated whole blood culture supernatant by conventional ELISA. Subjects were followed up on monthly basis to monitor the progression into disease. Of 13,163 persons only 309 subjects (3.6%) had converted to seropositive on either of the DAT or rK39 ELISA in one year interval. The percentage positivity of WBA and qPCR was equal in both seropositive (IGRA =19%, qPCR= 48.2%) and its control groups (IGRA =16.3%; qPCR= 42.8%) (p= 0.431; $\chi 2$ test). Of those IFN-γ positive persons, 63.8% and 60.7% persons remained IFN-γ positive over 1 year in seropositive and control groups respectively. The new incidence of IFN-γ positivity was 5.5%. Only one subject who was positive by both IGRA and qPCR at baseline developed into clinical VL. These findings confirm that SLA based IGRA is a promising tool for identification of clinically exposed immune individuals.

776

DETECTION OF *LEISHMANIA DONOVANI* AND *LEISHMANIA MAJOR* IN NATURALLY INFECTED SAND FLIES IN ISIOLO AND WAJIR COUNTIES, KENYA

Gladys C. Kerich

United States Army Medical Research Unit - Kenya, Kisumu, Kenya

Leishmaniasis has been termed as an emerging or re emerging disease in North Eastern Kenya. Over the past decade, several out breaks have been reported In Isiolo and wajir. While the causative agent, *Leishmania donovani*, has been isolated from the human so far, no work has been done to identify possible invertebrate hosts. Previous studies have pointed at *Phlebotomus orientalis* as the possible VL vector. However, natural infections have not yet been demonstrated. This study was designed to test sand flies from the area for natural *Leishmania* infection. Sampling was done between 2008-2011 in areas both in Isiolo and Wajir using CDC light traps baited with dry ice. Sand fly caught were stored in 70% ethanol and transported to the laboratory for processing. Ten percent of the collection was used for species identification while the rest (females) were pooled for DNA extraction and conventional PCR for detecting *Leishmania* infection (genus assay) and real time PCR for identifying the specific *Leishmania* species (species assay).A total of 1486 pools from Wajir and 2465 from Isiolo were tested, giving a total of 14078 and 30913 females respectively. Two pools from Wajir and three from isiolo tested positive for *L. donovani* while three additional pools from Isiolo tested positive for *L. major.* The minimum infection rates were .13% in wajir and 0.24% in Isiolo.The first report of naturally infected *L. donovani* and *L. major* in sand flies collected from both counties. The finding of *L. donovani* infected sand flies indicates that there is potential transmission of both Cuteneous leishmania (CL) and Visceral leishmania (VL) in the region. The finding of *L. major* positive sand flies raises questions on whether there are other vectors of *L. major* in Kenya apart from *P. duboscqi.*There is need for further investigation on the vector competence of the sand fly species of Isiolo and Wajir as well as a correlation with infection in human. Need for active surveillance in the region in light of political and civil unrest in neighboring *Leishmania* endemic countries.

777

HEALTHY HOUSING FOR HEALTHY LIVING: PARTICIPATORY DESIGN OF A HOUSING PROTOTYPE TO CONTROL VECTORIAL TRANSMISSION OF CHAGAS DISEASE IN LOJA PROVINCE, ECUADOR

Mario J. Grijalva[1], Claudia P. Nieto-Sanchez[1], Sylvia Jimenez[2], Guillermo Gomez[2], Darwin F. Guerrero-Jimenez[2], Esteban G. Baus[2]

[1]Ohio University, Athens, OH, United States, [2]Pontifical Catholic University of Ecuador, Quito, Ecuador

Chagas disease is caused by the parasite *Trypanosoma cruzi*, mainly transmitted by the feces of triatomine insects. Considering that triatomines find favorable environments in cracks of walls, floors, and roofs of poorly constructed houses, Chagas disease is deeply connected with life conditions present in rural areas of Latin America. Consequently and based on ten years of entomological research that shows constant triatomine reinfestation in the houses of Loja province in southern Ecuador, despite insecticide and educational interventions. We developed a *Healthy Housing Model* that considers the physical structure, disposition and organization of intra and peri-domicile as the main tool for long term Chagas disease control in the region. In order to guarantee social and cultural acceptability of the model, as well as communities' participation in the different phases of the project from decision-making to implementation and evaluation, we implemented a participatory research process based on positive deviance (PD) methodological framework. This process was aimed at identifying existing practices and knowledge in relation to houses' construction and insect protection, as well as attitudes in relation to locally available construction materials and improvement techniques. The descriptions and traditional knowledge shared through interviews, photo-voice and participatory sketching exercises, as well as housing infrastructure data collected from all the families were used to design a prototype that was designed with the communities in multiple sessions and adjusted by the architecture team to local communities' practices and aesthetics. Three different models of the prototype were developed and construction training was provided to community members to ensure technological transfer of the improvements implemented in the housing prototype. The prototype was constructed as a joint effort between the community and researchers, to serve as proof of concept prior to the possible scaling up of the intervention at the whole community.

778

AN EPIDEMIOLOGICAL MAP OF CUTANEOUS LEISHMANIASIS IN THE KINGDOM OF SAUDI ARABIA

Waleed S. Al Salem[1], Naomi Dyer[1], Emily Adams[1], Mohamed Alzahrani[2], Ziad Memish[2], Alvaro Acosta-Serrano[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]Saudi Ministry of Health, Riyadh, Saudi Arabia

Cutaneous leishmaniasis (CL) is the predominant vector-borne disease in the Kingdom of Saudi Arabia (KSA). Despite a significant reduction in the number of cases in recent years due to effective vector and rodent control initiatives, the uncontrollable urbanisation and the growing number of unresponsive patients to drug therapy are major concerns. Both *Leishmania major* and *L. tropica* are responsible for all CL cases in KSA, but their geographical distributions are unknown as patients are only clinically diagnosed. In this work, we report the molecular characterisation of the *Leishmania* spp from the main CL endemic regions in KSA. In addition, we describe the clinical features of the cutaneous lesions associated with each parasite species and patient responses to standardised drug treatments. Samples from a total of 104 adult individuals (89% male, 11% female) were collected from the regions of Riyadh, Algassem, Asir, Jazan, Al Ahsa, Al Madinah and Hail, which collectively represent ~80% of all reported cases of CL in KSA. Wound aspirations and skin biopsies were collected to identify parasite species and secondary infections, respectively. In addition, serum samples were taken to confirm leishmaniasis diagnosis using a novel ELISA method that measures levels of anti-alpha-galactosyl antibodies in CL patients. Results indicate 1) Using PCR-RFLP on the ribosomal internal transcribed spacer 1 region (ITS1), it was found that *L. major* is the main species causing CL in KSA. However, *L. tropica* is the only species found in the Southwest (Asir) and also in few cases from Al Madinah region. 2) Clinical presentations vary depending on the parasite species; ulcerated nodular lesions correlate with *L. tropica* infections and papular lesions are more frequent associated with *L. major* patients. 3) Interestingly, multiple lesions (up to 29 per patient) correlated only with *L. major* cases, whereas no more than 3 lesions were found in *L. tropica* patients. 4) Confirmed patients were referred for treatment with topical antifungals, followed by 1-2 courses of intralesional pentostam. Treatment response depends on several factors, including parasite species, clinical features, geographical location and the presence of secondary infections. Overall, ~82% of *L. major* cases responded to pentostam whereas 60% of *L. tropica* cases were resistant to the same drug. To our knowledge, this is the first epidemiological map of CL in the whole KSA.

779

PREVALENCE OF CHAGAS DISEASE IN BOLIVIAN IMMIGRANTS TO NORTHERN VIRGINIA

Rachel Marcus

LASOCHA, Washington, DC, United States

American Trypanosomiasis (Chagas disease) is thought of as an illness confined to Central and South America by most U.S.-trained physicians. With massive immigration of citizens from Chagas-endemic countries to the U.S. over the past 3 decades, the likelihood of significant disease burden is high, but at present estimated rather than formally assessed. There is a large Bolivian population in Northern Virginia, whose locality of origin is a high-risk area for for Chagas disease. We performed a pilot project of screening for Chagas disease in this population. A screening station was established on 2 occasions at a mobile consulate for the Bolivian embassy. All interested adults were screened with a rapid test kit (Chembio), and follow-up testing sent to the CDC for confirmatory ELISA (Wiener assay) and IFA. All participants were from the Cochabamba region. Of 24 screened patients, 9(38%)were positive by Chembio assay. Confirmatory assays were positive in 9/9 patients. Screening for Chagas disease in a high-risk Bolivian population suggests the high likelihood of significant disease burden in the Washington DC area. A more rigorous screening program in this population is warranted.

780

CLINICAL CARDIAC FINDINGS DESCRIBING DISEASE SEVERITY IN *TRYPANOSOMA CRUZI* INFECTED PERSONS IN A BOLIVIAN URBAN PUBLIC HOSPITAL

Emi Okamoto[1], Jacqueline Sherbuk[1], Gerson Galdos-Cardenas[2], Jeong Choi[2], Eva Clark[3], Omar Gandarilla[4], Lisbeth Ferrufino[5], Thomas Crawford[6], Rose Do[7], Antonio Fernandez[8], Jorge Luis Flores Franco[4], Robert Gilman[2], Caryn Bern[9]

[1]New York University School of Medicine, New York, NY, United States, [2]Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, United States, [3]University of Alabama School of Medicine, Birmingham, AL, United States, [4]Universidad Catolica Boliviana, Santa Cruz de la Sierra, Plurinational State of Bolivia, [5]Hospital Universitario Japones, Santa Cruz de la Sierra, Plurinational State of Bolivia, [6]University of MIchigan School of Medicine, Ann Arbor, MI, United States, [7]University of Colorado School of Medicine, Aurora, CO, United States, [8]Brown University School of Medicine, Providence, RI, United States, [9]University of California San Francisco, San Francisco, CA, United States

Trypanosoma cruzi, the parasite causing Chagas cardiomyopathy (CC), is the leading parasitic cause of morbidity and mortality in South America. Bolivia has the highest *T. cruzi* prevalence world-wide with infection rates of up to 43%. To evaluate ECG, echocardiogram, and serum-derived biomarkers of Chagas cardiomyopathy, we recruited 425 adults (48.7% male; mean age±SD, 57.5±12.7yrs) with and without *T. cruzi* infection from a large public hospital in Santa Cruz. We enrolled cardiac patients from the inpatient medicine service (n=128), cardiology clinic (n=94), and healthy controls (n=203). All patients underwent a cardiac history and demographic questionnaire for NYHA heart failure (HF) classification, blood draw, and ECG with QRS scoring. 250 patients also received an echocardiogram and 312 were chosen for serum biomarker analysis. Here we report clinical cardiac findings. By history, patients reported HTN (n=185, 43.4%), CAD (n=43, 10.1%), past pacemaker implantation (n=19, 4.5%), and HF symptoms (n=165, 38.8%). 324 patients (76.4%) were *T. cruzi* seropositive. For those in HF, no significant differences were noted in QRS score, EF, or left ventricular end diastolic diameter between seropositive and seronegative individuals. However, seropositive HF patients tended to have longer PR intervals (median 167 vs 153, IQR 153-192 vs 148-177), longer QRS duration (median 110 vs 102, IQR 97-149 vs 90-140), and lower heart rates (median 73 vs 86, IQR 65-92 vs. 69-94) than seronegative HF patients. All 9 cases of bradycardia in HF patients occurred in seropositive patients. Of seropositive individuals, characteristic Chagas EKG changes were more frequent in those with HF (n=134, 41.4%), with a significant difference ($p<0.001$) in the presence of atrial fibrillation, PVCs, right bundle branch block (RBBB), and left anterior fascicular block (LAFB). A hallmark Chagas finding, bifasicular block (RBBB with LAFB) was present in 13 seropositive individuals (4.2%). Forthcoming echocardiogram and serum biomarker results will provide a more in-depth evaluation of cardiac structural changes in this cohort and will help to determine reliable early indicators of CC risk. This would allow treatment to be targeted to patients with the highest likelihood of future morbidity and mortality.

781

SPATIAL ASSOCIATION BETWEEN *TRYPANOSOMA CRUZI*-INFECTED *TRIATOMA INFESTANS* AND *T. CRUZI*-SEROPOSITIVE DOGS IN AREQUIPA, PERU

Ricardo Castillo Neyra[1], Lily Chou Chu[2], Monica Llamosas Chu[2], Victor Quispe-Machaca[2], Jenny Ancca-Juarez[2], Fernando S. Malaga Chavez[3], Cesar Naquira[2], Robert H. Gilman[1], Michael Z. Levy[4]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Universidad Peruana Cayetano Heredia, Lima, Peru, [3]Dirección Regional del Ministerio de Salud, Arequipa, Peru, [4]Center for Clinical Epidemiology and Biostatistics - Department of Biostatistics and Epidemiology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, United States

Chagas disease, a vector-borne disease transmitted by triatomine bugs and caused by the parasite *Trypanosoma cruzi,* affects 8 - 10 million people in the Americas. Indoor residual spraying campaigns are the most effective interventions to stop the transmission of the parasite and are routinely conducted in Arequipa, Peru to eliminate *Triatoma infestans*, the only vector in this area. Dogs are important reservoirs of *T. cruzi*; however, they have not been included as targets for interventions to halt the life cycle of the parasite. The objectives of this study were (i) to determine the seroprevalence of dogs in an area with re-emerging *T. infestans* following insecticide control; (ii) to describe the spatial distribution of dogs, *T. infestans* and *T. cruzi*; and (iii) to determine if proximity to infected triatomines is associated with higher risk of infection with *T. cruzi* in dogs. We conducted a cross-sectional serological screening to detect antibodies against *T. cruzi* in dogs, an entomological survey to collect vectors from households. We assessed spatial clustering of seropositive animals and infected vector colonies. Canine seroprevalence in the area was 13.0% (n=154). Infected colonies showed spatial clustering evaluated with K-function difference between 20 and 75 meters in constrast with seropositive dogs which did not show spatial clustering. The spatial intensity of all captured colonies and all sampled dogs evaluated with quartic kernels was homogeneous over the study area, but the spatial intensity of infected colonies and seropositive dogs showed zones with higher intensity and these areas overlap in most part. The adjusted odds ratio between seropositivity to *T. cruzi* and being close to an infected triatomine (defined as ≤50m) was 5.67 (95%CI: 1.12 – 28.74; p=0.036) after adjusting for age and sex of the dog. Interventions based on entomological data that are used to determine high-risk areas for humans could include the presence of dogs around houses where infected triatomines were collected to prevent future resurgence of the parasite.

782

MOLECULAR CHARACTERIZATION OF MILTEFOSINE UNRESPONSIVENESS IN *LEISHMANIA DONOVANI*

Puja Tiwary, Dinesh Kumar, Madhukar Rai, Shyam Sundar

Institute of Medical Sciences, Varanasi, India

Drug resistance is a major problem in leishmaniasis chemotherapy. Pentavalent antimonials have been the first-line drugs in the treatment of all forms of leishmaniasis for more than 70 years. But during last 20 years various cases of SbV resistance have been reported. There was need of some novel and effective antileishmanial drugs raised and thus miltefosine, paromomycin and amphotericin B used as replacement of SbV. During recent years these novel drugs also showed unresponsiveness in various parts of world. Understanding molecular mechanism behind the drug resistance is very crucial for success of such antileishmanial drugs. Genomic analysis has been performed primarily with SAG resistant *Leishmania* species and here we investigate molecular alterations in Miltefosine resistance in *L. donovani*. RNA expression profiling using microarrays is a suitable approach to study such events leading to a drug-resistance phenotype. We selected clinically Miltefosine unresponsive and responsive population of *L. donovani* promastigotes. Gene expression of both type of strains were studied using RNA microarrays. Genes having more than two fold of variation in expression used to validated by real-time RT-PCR. RNA expression profiling of Miltefosine unresponsive *L. donovani* revealed the over and down expression of few genes involved in drug resistance. Further study is going on for the validation of resistance molecular markers.

783

A PROTOCOL TO OPTIMIZE MICROSATELLITE DNA AMPLIFICATION OF *TRYPANOSOMA BRUCEI GAMBIENSE* FROM BODY FLUIDS

Jacques Kaboré[1], Thierry De Meeûs[2], Annette MacLeod[3], Hamidou Ilboudo[1], Paul Capewell[3], Mamadou Camara[4], Adrien Marie Gaston Belem[5], Adrien Marie Gaston Belem[5], Bruno Bucheton[2], Vincent Jamonneau[2]

[1]CIRDES URBIO, Bobo-Dioulasso, Burkina Faso, [2]IRD-CIRDES, Bobo-Dioulasso, Burkina Faso, [3]Wellcome Centre for Molecular Parasitology, University of Glasgow, Biomedical Research Centre, Glasgow, United Kingdom, [4]Programme National de Lutte contre la Trypanosomose Humaine Africaine, Conakry, Guinea, [5]Université Polytechnique de Bobo-Dioulasso, Bobo-Dioulasso, Burkina Faso

Microsatellite genotyping of *Trypanosoma brucei gambiense*, the causative agent of human African trypanosomiasis or sleeping sickness, and population genetics tools, are useful for inferring population parameters such as population size and dispersal. Amplifying parasite DNA directly from body fluids (i.e. blood, lymph or cerebrospinal fluid) allows avoiding costly and tedious isolation phases. It is however associated to increased frequencies of amplification failures (allelic dropouts and/or null alleles). We present a study focused on improving microsatellite *loci* amplification of *T. brucei gambiense* from Guinean sleeping sickness foci. We checked for the real nature of blank and apparent homozygous genotypes of parasite DNA directly amplified from body fluids. We tested the effect of three different DNA quantities for different microsatellite *loci* of trypanosomes from different body fluids. Our results show that some initially blanks and homozygous genotypes happen to be actual heterozygous genotypes. In Guinea, lymph from the cervical nymph nodes, known to contain the highest concentrations of parasites, appeared to provide the best amplification results. Simply repeating the PCR may be enough to retrieve the correct genotype, but we also show that increasing initial DNA content provides better results while undertaking first amplification. We finally propose an optimal protocol for amplifying *T. brucei* DNA directly from body fluids that should be adapted to local characteristics and/or constraints.

784

ANTI-*LEISHMANIA DONOVANI* ANTIBODIES ENHANCE PROMASTIGOTES INTERNALIZATION INTO HOST MACROPHAGE

Abdalla H. Sharief[1], Sara Mohamed[2], Eltahir Khalil[3], Ahmed Musa[4], Samia Omer[1], Ahmed El-Hassan[1]

[1]Tropical Medicine Research Institute, National Centre for Research, Khartoum, Sudan, [2]Central Laboratory, Khartoum, Sudan, [3]Institute of Infectious Diseases, University of Khartoum, Khartoum, Sudan, [4]Institute of Endemic Diseases, University of Khartoum, Khartoum, Sudan

Leishmania spp. promastigotes preferentially infect host macrophages, where parasite internalization is facilitated by several host and parasite surface molecules. This study aimed to demonstrate the role of humoral immunity in *Leishmania* parasite internalization into host macrophages. First, informed consent sera were obtained from 67 parasitologically confirmed visceral leishmaniasis patients reporting to our field treatment centre, Eastern Sudan. Then following titre determination, sera that had a titre of >102,400 were selected for parasite coating. An *in vitro* parasite internalization system was developed to enhance the *Leishmania*/macrophage interactions. The mean parasite number per monocytes was

626 ± 91 for antibody-coated *Leishmania donovani*, compared to 412 ± 70 uncoated isolates (p= 0.01). On the other hand, the percentage of infected cells was significantly higher for all antibody-coated isolates (100%) compared to uncoated ones (40%). This evidence of high infectivity probably points to the fact that anti-*Leishmania* antibodies facilitated the parasite uptake by host macrophages and monocytes-derived macrophages (MDM). Moreover, the rate of parasite uptake by MDM was significantly higher compared to monocytes (p= 0.00). This could be explained by the fact that the functional capabilities of fully differentiated macrophages differ from monocytes. In conclusion, host humoral immunity probably plays a pivotal role in *Leishmania* parasites internalization into host macrophages.

785

HIGHER PARASITE BURDEN IN HEALTHY (ASYMPTOMATIC) INDIVIDUALS CONTRIBUTE IN PROGRESSION OF VISCERAL LEISHMANIASIS

Abhishek K. Singh, Medhavi Sudarshan, Ankita Chourasia, Madhukar Rai, Jaya Chakravarty, Shyam Sundar

Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

In an area endemic for Visceral Leishmaniasis (VL), subclinical or asymptomatic infections play a crucial role in progression of disease. We determined the parasite load by quantitative PCR (qPCR) in healthy infected population living in an area endemic for Visceral Leishmaniasis. We enrolled 13366 persons from 11 villages of highly endemic region of Bihar, India. We conducted two sero- surveys with 1 year time interval for identification of incident infected healthy individuals. Parasite load using TaqMan based qPCR were done on these sero-converted individuals and its matched control populations. Individuals having parasite load greater than 1 genome/ml of blood was considered as positive by qPCR. Follow-up visit to the homes of each individual were made to monitor the disease conversion in this cohort. Agreements between seroconversion and qPCR were accessed by kappa value. Total 235 persons were converted their serology within 12 month intervals. Of these 235 sero-converters 105 (44.6%) individuals were also positive by qPCR. However, similar number of controls groups (87/ 237, 37%) also showed positivity by qPCR. The agreement between sero-converter and qPCR was moderate. Among all individuals only one were converted into disease that has parasite load 146 parasite genome/ ml of blood. These findings suggest the usefulness of parasite load in healthy individuals living in an endemic area of Bihar and contribute as a good tool for VL elimination programme.

786

PHENOTYPIC AND FUNCTIONAL EXAMINATION OF NEUTROPHILS FROM BRAZILIAN CUTANEOUS LEISHMANIASIS PATIENTS

Richard E. Davis[1], Jacilara Alexandrino[2], Pedro Paulo Carneiro[2], Olivia Bacellar[2], Edgar M. Carvalho[2], Mary Wilson[1]

[1]University of Iowa, Iowa City, IA, United States, [2]Federal University of Bahia, Salvador, Brazil

The protozoan parasite *Leishmania braziliensis* is the causative agent of the disease cutaneous leishmaniasis (CL) which can cause ulcerated skin lesions in individuals living in endemic regions. Skin pathology is thought to result from overabundant inflammatory myelocytic and lymphocytic cell responses. The effect of acute CL infection on neutrophil (PMN) mobilization and activation in the peripheral blood and how PMN might influence other cells of the immune response during disease remains unclear. In collaboration with investigators from the Federal University of Bahia (UFBA) in Salvador, state of Bahia, Brazil, we studied PMN from peripheral blood of patients with acute CL encountered in a rural leishmania-treatment center in Corte de Pedra, Bahia. We hypothesized that, as in other acute inflammatory models, peripheral blood PMN from CL patients would contain increased numbers of activated PMN with increased production of reactive oxygen species and the ability to suppress T lymphocyte responses. We detected PMN activation by higher CD66b and CD11b, and lower CD62L expression according to flow cytometry. We also detected a potentially suppressive, CD66b+ low-density PMN (LD-PMN) population in the PBMC fraction following centrifugation in a density gradient. Superoxide production was detected by spontaneous and PMA-induced ferricytochrome C reduction. T cell responses were detected by *in vitro* stimulation of peripheral blood mononuclear cells (PBMCs) with parasite antigen, followed by surface staining for Ki67 (proliferating cells) and intracellular staining for IFNγ when stimulated. Contrary to our hypothesis, we found similar levels of surface CD66b and CD11b and similar superoxide production in both CL patients and controls, but decreased CD62L in CL patients, indicating a difference in some, but not all measures of PMN activation. We detected a population of potentially suppressive CD66b+ LD-PMN in the PBMC fraction of CL patients. However, co-incubation of PMN of normal density with PBMCs resulted in enhanced, rather than suppressed, T cell responses. Surprisingly, a population of PMN with increased expression of the MHCII complex HLA-DR was observed in CL patients. We conclude that human peripheral blood PMN are not suppressors of T cell responses in subjects with CL. Ongoing studies will determine whether the unique PMN subset expressing HLA-DR is capable of presenting antigen to T cells during human CL.

787

PARASITOLOGICAL CONFIRMATION OF ASYMPTOMATIC INFECTION IN ENDEMIC AREAS FOR CUTANEOUS LEISHMANIASIS IN COLOMBIA

Mariana Rosales Chilama

CIDEIM, Cali, Colombia

Asymptomatically infected individuals in endemic areas for cutaneous leishmaniasis (CL) can be a hidden and large reservoir of parasites available for anthropophilic vectors. Viable *Leishmania* persist indefinitely after clinical resolution of disease. Whether parasites persist during asymptomatic infection and its impact on disease transmission is unknown. We aimed to parasitologically confirm asymptomatic infection and the relative parasite burden in mucosal tissues and peripheral blood of inhabitants of communities endemic for CL in Colombia. Individuals from 4 communities in Nariño and Risaralda were included. Participants were categorized as uninfected, asymptomatically infected; healed CL or active CL. Blood monocytes, lesion and scar aspirates, and nasal mucosa, conjunctiva and tonsil swabs were obtained from participants. Detection of L. Viannia kDNA was performed by PCR, and parasite viability confirmed by 7SLRNA RT-qPCR In communities of Risaralda the population was predominantly represented by individuals with history of CL (67%). kDNA positivity was found in 43% of individuals. Samples with the highest frequency of kDNA positivity were blood monocytes (37%) and nasal swabs (15%). Communities in Nariño were largely composed of uninfected individuals (63%) followed by those with history of CL (25%) and asymptomatic infection (9%), of which 27% were kDNA positive; swabs of tonsil (24%) and nasal mucosa (15%) were the most frequently positive samples. Samples with highest parasite burden were tonsil and nasal mucosal swabs Parasite persistence in individuals without clinical signs of infection highlights their potential in transmission. High frequency of kDNA positive blood samples in individuals with history of CL supports their previously unrecognized role as a source of parasite acquisition by the vector. Parasite detection in mucosal tissues may identify potential risk of disease activation. Understanding of parasite persistence in asymptomatic infection and healed CL in transmission is needed for the development of community-targeted control strategies.

788

GLOBAL METHYLATION CHANGES IN THE HEART DURING THE ACUTE INFECTION IN A CHAGAS RAT MODEL

Edgar A. Florentini, Noelia P. Angulo, Holger Mayta, Manuela R. Verastegui, Robert H. Gilman

Universidad Peruara Cayetano Heredia, Lima, Peru

Chagas disease, caused by the protozoan *Trypanosoma cruzi*, is the most costly parasitic disease of the Americas in terms of morbidity and mortality. In one third of infected patients, it produces severe and progressive injury of the heart, nearly always associated with a fatal outcome. The mechanisms of interaction, invasion, and pathogenesis in this disease are not only extremely complex, but also not fully understood. Genome methylation, a subarea in the study of epigenetics, has recently showed relevant information about distinct pathogenic scenarios, showing modulations as a consequence of inflammation, cancer, infection, fibrosis, among others. In this study we aimed to detect cardiomyocyte genome methylation levels modulations as a consequence of the heart injury during the acute stage of infection of T.cruzi in a rat model. 8 Holtzman-strain, 6-8 week old male rats were peritoneally inoculated with 107 metacyclic trypomastigotes, "Arequipa" strain, and sacrificed at 36 days post infection. 8 rats of the same strain were not infected and served as controls. Active infection was interrogated by microhematocrit method, PCR, and anti-IgG TESA-ELISA. The heart was removed immediately after death and fixed in formalin 3.7% formaldehyde in PBS. Methylation status was examined by semiquantitative, immunohistochemical evaluation of whole heart sections using a monoclonal antibody against 5-methylcytosine, considering that a direct relationship exists between genome methylation levels and intensity of the nuclei staining. At least 100 cardiomyocyte nuclei were blindly examined. All (8/8) rats presented a decrease in the levels of cardiomyocyte nuclei intensity, compared to controls ($P<0.05$). All methods of diagnosis confirmed the infection in all inoculated subjects. Our results indicate that as a consequence of the acute infection of *T. cruzi*, a significant decrease of genome methylation levels occurs in affected heart myocytes *in vivo*.

789

A CONGENITAL RAT MODEL OF CHAGAS DISEASE

Noelia Angulo[1], Edgar-Alejandro Florentini[1], Janet Acosta[1], Ines Cabello[1], Manuela Verastegui[1], Robert Gilman[2]

[1]Infectious Diseases Research Laboratory-Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Johns Hopkins University-Bloomberg School of Public Health, Baltimore, Peru

Trypanosoma cruzi is responsible for Chagas Disease. Usually, the infection occurs in the childhood, and most cases are evident in the chronic phase, when irreversible cardiac damage is diagnosed, may inexorably lead to death. To the present date there are unanswered questions about the mechanism of *T. cruzi* to cross placental barrier and infect the fetus. Additionally, there is a lack of a good diagnostic test in order to start early the treatment of the positive newborns to congenital Chagas, which is of vital importance, because benznidazole and nifurtimox are effective only in the acute phase of the disease. Our aim was develop an animal model for congenital Chagas which could help to the research. Six female Holtzman rats were inoculated by intraperitoneal route with 10^7 blood trypomastigotes from Arequipa isolate; control group was inoculated with saline. Three days after the start of the mating period, females were inoculated. The observation of a vaginal plug determined the pregnancy day one. Parasitemia was evaluated at 10 and 19 days post inoculation (dpi). Necropsy was performed over 19 dpi, blood samples of the pups were evaluated by microconcentration technique, serology and molecular test. Placenta and umbilical cord were collected and immersed in absolute ethanol; also, placenta was collected in phosphate buffer solution pH 7,2 for histopathological analyses. Parasitaemia of the mothers peaked at 10dpi with a media of log 3.4 parasites/ml, while at 19dpi it decreased to a media of log 3.4 parasites/ml. Of 44 neonates analyzed so far, which were born to an infected mother, 70.45% (31 of 44) were positive for the Arequipa strain using PCR/qPCR. Random samples of this Arequipa group were subject to further analysis by histology; amastigote nests were found in 28.57% (4 of 14) respective placentas. Similarly, 33.33% (7 of 21) neonate blood clot samples were positive for anti-TESA IgM antibodies by ELISA, and 4.55% (2 of 44) neonates exhibited *parasitemia* in their amniotic fluid. Our experimental model of congenital Chagas transmission allowed us to obtain infected as well as no infected pups samples from the same mother, which is a great advance to perform studies to improve the know ledge about routes of transmissionand allowing the evaluation of diagnosis techniques as an alternative to the human studies and the troubles and costs of what that implies.

790

MODULATION OF ACTIVATION-ASSOCIATED MICRORNA ACCUMULATION DURING MONOCYTE-TO-MACROPHAGE DIFFERENTIATION

Renee L. Eigsti[1], Mary E. Wilson[2], Joel W. Graff[3]

[1]University of Iowa Carver College of Medicine, Iowa City, IA, United States, [2]University of Iowa Carver College of Medicine, Departments of Internal Medicine, Microbiology and Epidemiology, and the Veterans Affairs Medical Center, Iowa City, IA, United States, [3]University of Iowa Carver College of Medicine, Department of Internal Medicine and the Veterans Affairs Medical Center, Iowa City, IA, United States

Circulating monocytes recruited to tissues can become monocyte-derived macrophages (MDMs). Monocytic cell gene expression programs are influenced by environmental cues such as cytokines and cell-cell interactions. The regulation of microRNA (miRNA) expression in response to these environmental cues is not as well characterized. We recently reported high basal expression of several activation-associated miRNAs in primary human MDMs relative to freshly isolated peripheral monocytes. This observation suggests that accumulation of key miRNAs occurs as part of monocyte-to-macrophage development. We hypothesized that the rate of activation-associated miRNA accumulation could be modified in monocytic cells responding to environmental stimuli during differentiation. Indeed, the rate of miR-193b and miR-222 accumulation was augmented by IL-4 treatment. While LPS stimulation augmented miR-146a and miR-155 expression, the accumulation miR-125a-5p and miR-222 was antagonized by IFN-beta or IFN-gamma. Toward determining a mechanism underlying activation-associated miRNA accumulation, we found that expression of primary miRNA, not Dicer, directly correlated with activation-associated miRNA expression. Interestingly, miR-155 and miR-193b accumulation was greater in MDMs differentiated within total PBMC cultures than as purified monocyte cultures. Both of these miRNAs were also highly expressed in purified monocytes co-cultured with non-monocytic PBMCs. In summary, the rate of activation-associated miRNAs accumulation during monocyte-to-macrophage differentiation can be modified not only through defined stimuli such as LPS and cytokine treatments but also through an uncharacterized interaction between monocytic and nonmonocytic cells. Biologically-important miRNAs in macrophage differentiation and activation will be assessed for the capacity to polarize macrophages towards a M1 phenotype that induces microbicidal responses toward intracellular pathogens such as *Leishmania*.

791

INFECTIVITY STUDY OF THREE RODENTS HOSTS OF *LEISHMANIA (VIANNIA) BRAZILIENSIS*: POSITIVITY, PARASITE LOAD AND TISSUE TROPISM

Ricardo A. Zampieri[1], Carloina L. Jorge[1], Maria Fernanda L. da Silva[1], Marai Sandra Andrade[2], Sinval P. Brandao-Filho[2], Lucile M. Floeter-Winter[1], **Jeffrey J. Shaw**[1]

[1]São Paulo University, Sao Paulo, Brazil, [2]Centro de Pesquisas Aggeu Magalhães, Recife, Brazil

For over three decades, the primary reservoirs of *Leishmania (Viannia) braziliensis* were not known, but the development of PCR protocols to detect parasites in samples obtained from sylvatic animals implicated some vertebrate species such as rodents and marsupials as primary reservoirs of *L. (V.) braziliensis*. To observe the infection profile in some of these reservoirs, animals from established colonies of *Nectomys squamipes*, *Necromys lasiurus* and *Rattus rattus* were experimentally infected with 10^6 promastigote forms of *L. (V.) braziliensis*. After six weeks of infection, samples of skin, spleen and liver were processed to purify template DNA for detection and quantification PCR assays, using DNeasy Blood and Tissue kit (Qiagen), according to manufacturer's protocol. The initial detection protocol consisted in a nested PCR assay using two pairs of SSU rDNA (Small Subunit Ribosomal gene) derived oligonucleotides. The first PCR used primers that amplify a conserved region in all trypanosomatids and the second reaction used primers that amplify a common region of the *Leishmania* genus. The quantification protocol consisted in a real time SYBR-Green PCR, wherein the parasite load was estimated by normalizing the number of SSU rDNA copies per host glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) copy number. We observed a higher positivity in samples of *N. lasiurus* and *N. squamipes* indicating that these species are more susceptible than *R. rattus*. However, all samples presented a low parasite load, 11.4, 128 and 235 SSU rDNA copies in *R. rattus, N. squamipes* and *N. lasiurus* samples, respectively, what correspond to the DNA of at most one parasite/50 ng of host DNA. Besides, we were not able to observe parasite tissue tropism for the three analysed species. The high positivity in samples of *N. lasiurus* and *N. squamipes*, and concomitant low parasite load, may be features that support the life cycle of the parasite, ensuring the role of these rodents as reservoirs in sylvatic cycle.

792

IS OVER-DEPENDENCE ON MALARIA RDTS DISGUISING MORBIDITY DUE TO OTHER DEADLY HEMOPARASITES AT PERIPHERAL HEALTH FACILITIES?

Elizabeth M. Chandra[1], Yewubdar Mandefro[1], Shiret Yinges[2], Belete Timerga Chere[3]

[1]Addis Continental Institute of Public Health, Addis Ababa, Ethiopia, [2]ICAP Ethiopia, Addis Ababa, Ethiopia, [3]Addis Ababa University, Addis Ababa, Ethiopia

Microscopy and multispecies malaria RDTs are standard laboratory tests for febrile patients suspected of having hemoparasites at district health centers and rural health posts, respectively, in Ethiopia. Significant number of malaria and relapsing fever (*Borrelia recurrentis*) cases are reported among seasonal migrant workers during major malaria transmission season in endemic areas. A cross-sectional study was conducted to compare the laboratory diagnosis and treatment of outpatient febrile patients at 2 district health centers and 11 satellite rural health posts during the major malaria transmission season in central Ethiopia. Demographic, clinical and laboratory data were abstracted from the monthly morbidity reports of each facility. Laboratory tests were done for 2326 and 711 patients in district health centers and health posts, respectively. 170 (5.6%) of all total febrile patients were migrant seasonal workers of which 106 (62.4%) were seen at the health centers. Prevalence of malaria at health posts was 13.9% (99/711): 53 *P. falciparum* infection, 44 non-*falciparum* malaria and 2 mixed infections. Prevalence of malaria at health centers was 9.3% (217/2326) of which 97 (44.7%) *Plasmodium falciparum*, 112 (51.6%) *P. vivax*, and 8 (3.7%) mixed infections. The overall prevalence of *B. recurrentis* infection was 1.4% (33/2326), of which 12 were migrant workers making the subgroup prevalence 11.3% (12/106). In conclusion, detection and species identification of plasmodium infections by both microscopy and multispecies RDTs tests, and relapsing fever by microscopy guided rational drug administration at peripheral health care facilities. *B. recurrentis* is an important etiologic agent of febrile illness in the region. However, non-reporting of relapsing fever at the health posts can be attributed to diagnostic limitation with risk of missed diagnosis of the highly contagious and potential life-threatening pathogen. Diagnosis and treatment algorithms for febrile patients in endemic areas of multiple etiologies such as plasmodia and Borellia should take in to consideration epidemiology and seasonality. More systematic study involving microscopy-RDT parallel testing and monitoring of treatment outcomes during major transmission seasons might shade more light on the extent of the problem.

793

Γ-RAY POLYMERIZED NANOSPHERES FOR SEROLOGICAL DIAGNOSIS OF MALARIA

Hiroyuki Oku[1], Kazuhiko Yano[2], Brian Bacay[3], Shinya Kitamura[1], Mai Fukuno[1], Keiichi Yamada[1], Elena A. Villacorte[3], Pilarita T. Rivera[3], Shigeyuki Kano[2]

[1]Department of Science and Engineering, Gunma University, Gunma, Japan, [2]Research Institute, National Center for Global Health and Medicine, Tokyo, Japan, [3]College of Public Health, University of the Philippines Manila, Ermita, Manila, Philippines

Malaria is a major public health problem especially in tropical and sub-tropical regions of the world. The methods most commonly used to diagnose malaria patients sera, such as IFAT and ELISA, are not easy methods to examine in the bedside and the clinical laboratory in a hospital. Here, we wish to report the development of novel peptide immobilized polymer nanospheres for the detection of specific antibodies in malaria patients sera. The peptide antigens (~20 residues) were designed from two *Plasmodium* specific sequences in lactate dehydrogenase (pLDH) and in enolase (AD22). Each test material is prepared by chemical coupling of peptides to the surface of the diethylene glycol dimethacrylate and methacryloyl-OSu copolymer nanosphere. At the beginning of agglutination test, serum samples were 2-fold serially diluted (1/16···1/16384) with phosphate buffered saline in simple 96-well microplates with U-shaped bottom. Then, the nanospheres suspension was added to the microplates, which were followed by vigorous agitation. In the microplates, aggregation patterns can be observed almost at 4-6 h after the reaction, when left at room temperature. We have compared the reactivity against malaria patients' sera collected in endemic regions of the Philippines: (a) Mixed infected-, (b) *falciparum*-, (c) *vivax*-malaria patients, and (d) feverish patients (malaria negative). Successful results were observed for the pLDH material, which showed good specificity for three kinds of malaria patients compared with feverish patients. Interestingly, in the case of AD22 material, malaria patients did not show distinct titer values from feverish patients. This is due to the difference of antibody persistence against enolase (AD22) and lactate dehydrogenase (pLDH) in the endemic area where residents are sequentially infected by *Plasmodium* parasites. Therefore, these results probably indicates that pLDH and AD22 antigens are suitable to detect present and recent-past infections, respectively.

794

EVALUATION OF THE MALARIA RAPID DIAGNOSTIC TEST VIKIA MALARIA AG PF/PAN™ IN ENDEMIC AND NON-ENDEMIC SETTINGS

Stéphane Picot[1], Boubacar Traore[2], Daniel Eibach[1], Boubacar Coulibaly[2], Nianégué Coulibaly[2], Mourad Bouchrik[3], Fanta Siby[4], Anne-Lise Bienvenu[1]

[1]Malaria Research Unit, Lyon, France, [2]Centre de Santé de Référence Commune VI, Bamako, Mali, [3]Laboratoire de Parasitology and Mycology, Hôpital Militaire Mohamed V, Faculty of Medicine, Rabat, Morocco, [4]Direction Régionale de la Santé, Ministry of Health, Bamako, Mali

Malaria rapid diagnostic tests (RDTs) are a useful tool in endemic malaria countries, where light microscopy is not feasible. In non-endemic countries they can be used as complementary tests to provide timely results in case of microscopy inexperience. This study aims to compare the new VIKIA Malaria Ag Pf/Pan™ RDT with PCR-corrected microscopy results and the commonly used CareStart™ RDT to diagnose *falciparum* and non-*falciparum* malaria in the endemic setting of Bamako, Mali and the non-epidemic setting of Lyon, France. Blood samples were collected during a 12-months and six-months period in 2011 from patients suspected to have malaria in Lyon and Bamako respectively. Discordant results were corrected by real-time PCR. Samples of 877 patients from both sites were included. The VIKIA Malaria Ag Pf/Pan™ had a sensitivity of 98% and 96% for *Plasmodium falciparum* in Lyon and Bamako respectively, performing similar to PCR-corrected microscopy. The VIKIA Malaria Ag Pf/Pan™ performs similar to PCR-corrected microscopy for the detection of *P. falciparum*, making it a valuable tool in malaria endemic and non-endemic regions.

795

PREVALENCE OF ASYMPTOMATIC MALARIA INFECTIONS IN COLOMBIAN REGIONS WITH DIFFERENT EPIDEMIOLOGICAL PROFILES

Andres Vallejo[1], Juan P. Quintero[1], Juan Gutierrez[2], Alvaro Alvarez[1], Yoldy Benavides[1], David Forero[1], Julian Muñoz[1], Myriam Arévalo-Herrera[3], Socrates Herrera[1]

[1]Malaria Vaccine and Drug Development Center (MVDC), Cali, Colombia, [2]University Of Georgia, Athens, GA, United States, [3]Malaria Vaccine and Drug Development Center (MVDC) and School of Health, Universidad de Valle, Cali, Colombia

This study evaluated the prevalence of *Plasmodium falciparum* and *P. vivax* asymptomatic infections in three different malaria regions of Colombia with different epidemiological profiles: Tierralta (Ta), Tumaco (Tu) and Buenaventura (Bv). We conducted cross sectional surveys in 8 sentinel sites in which 1,170 subjects from 267 households were studied. Participants were asked to respond to a Knowledge, Attitudes and Practices (KAP) questionnaire and then were bled to determine the prevalence of malaria infection and sero-prevalence. Malaria diagnose was carried out using blood thick smears (TS) and RT-qPCR, whereas serology was assessed by IFAT and ELISA. The KAP survey indicated that besides high knowledge scores in the three communities, there are gaps in practices such as treatment adherence and use personal protection measures. In addition, we found negative correlation between socio-demographic scores and the seropositivity (PvMSP-1) with P value of 0.025. Whereas the overall prevalence of asymptomatic infection measured by TS was ~1%, by RT-qPCR it was 6.4% (n=45), with a greater proportion (21%) in 40-50 years old individuals. Furthermore different regions displayed different prevalence of asymptomatic infections: Bv 12%, Ta 1% Tu 4.3%. From these 45 samples, 66.2% were positive for *P. vivax* and 33,8% for *P. falciparum*, which correlates to the overall parasite prevalence in Colombia. While IFAT serology indicated greater recognition of *P. falciparum* (53%) than of *P. vivax* (18%), ELISA analyses indicated that 63% percent of reactivity to *P. vivax* (40%= MSP-1 and 39% PvCS) with 17% of double positives correlating with previous malaria episode of the population (~50%). We found highest reactivity index (51%) in Tu, concordant with the high malaria incidence, followed by Ta (49%) and Bv (19%). This study strengthen the importance in conducting active case surveillance as mean to more accurately determine malaria incidence, as well as to eliminate malaria reservoirs, and the need to use technique more sensitive than TS.

796

WILL CAREGIVERS OF UNDER-FIVE CHILDREN IN RURAL GHANA ACCEPT TEST-BASED MANAGEMENT OF MALARIA?

Frank Baiden[1], Seth Owusu-Agyei[1], Eunice Okyere[1], Mathilda Tivura[1], George Adjei[1], Daniel Chandramohan[2], Jayne Webster[2]

[1]Kintampo Health Research Centre, Kintampo, Ghana, [2]London School of Hygiene & Tropical Medicine, London, United Kingdom

The shift to test-based management of malaria (TBMM) is a departure from the presumptive approach that caregivers of under-five children in high transmission settings have been used to for many years. We used a survey with logistic regression analysis to explore the determinants of the willingness of caregivers in rural Ghana to accept TBMM. We followed it up with focus group discussions to explore the major emergent issues. A total of 3047 caregivers were interviewed. Nearly all (98%) reported a preference for TBMM over presumptive treatment. Caregivers who preferred TBMM were less likely to be concerned about the denial of ACT to their test-negative children (O.R. 0.57, 95% C.I. 0.33 -0.98). Caregivers who had valid (adjusted O.R. 1.30, 95% CI 1.07-1.61) or expired (adjusted O.R. 1.38, 95% CI 1.12-1.73) insurance cover were more likely to be concerned about the denial of ACT to their RDT-negative children than caregivers who had never secured national health insurance cover. Perception that a blood test at health centre level represents improvement in the quality of care, leads to improvement in treatment outcomes, and offers opportunity for increased communication between health workers and caregivers are factors that promote acceptability. Acceptability is also enhanced by engaging caregivers in the procedures of the test. Apprehensions about negative health worker attitude could however undermine acceptance. The high acceptability of test-based management of malaria among caregivers in this population is because of the expectation that it will lead to improvement in the quality of care.

797

MALARIA CASE MANAGEMENT IN PAPUA NEW GUINEA PRE- AND POST-IMPLEMENTATION OF A REVISED TREATMENT PROTOCOL

Justin Pulford[1], **Brown Kaupa**[1], Serah F. Kurumop[1], Ivo Mueller[2], Peter M. Siba[1], Manuel W. Hetzel[3]

[1]Papua New Guinea Institute of Medical Research, Goroka, EHP, Papua New Guinea, [2]Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, [3]Swiss Tropical and Public Health Institute, Basel, Switzerland

This study aimed to document malaria case management practices in Papua New Guinea pre- and post-implementation of a revised national malaria treatment protocol. The revised protocol stipulates routine testing of malaria infection by rapid diagnostic test (RDT) or microscopy, anti-malarial prescription to test positive cases only, and the introduction of artemether-lumefantrine (AL) as the first-line anti-malarial. The study hypothesized that the availability of malaria RDTs and AL and their use would increase over time, whilst the overall number of anti-malarial prescriptions would decrease. Data were collected via three countrywide cross-sectional surveys of randomly selected health facilities in 2010 (pre-implementation), 2011 (during implementation) and 2012 (12 months post implementation). Collectively, a total of 255 health facilities were surveyed across this time and 1568 malaria case management patients were observed. All data were collected using structured survey instruments. Only preliminary analyses were completed at the time of drafting this abstract. These data indicate a substantial increase in the

availability of malaria RDT or microscopy between 2010 and 2012 (15.2% and 53.4%, respectively) and a similar increase in the availability of AL (0%, 51.5%, respectively). In 2010, only 18.6% of observed febrile patients had a malaria RDT or blood slide taken, although 96.4% were prescribed an anti-malarial. Comparative data from 2012 have yet to be analysed. It is anticipated that the resulting findings, due to be completed in June 2013, will provide a clear indication as to the availability of diagnostic and treatment resources required for implementation of the new protocol as well as the level of health worker adherence to it.

798

IMPROVING QUALITY CONTROL OF THE MICROSCOPIC DIAGNOSIS OF MALARIA IN SENEGAL, 2009-2011

Ibrahima Diallo[1], Julie Thwing[2], Babacar Faye[3], Michael Aidoo[2], Moustapha Cisse[1], Alioune Badara Gueye[1], Medoune Ndiop[1], Fatou Ba Fall[1], Mady Ba[1]

[1]National Malaria Control Program, Dakar, Senegal, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Universite Cheikh Anta Diop, Dakar, Senegal

Blood film microscopy is considered the gold standard for diagnosis of malaria, but is dependent on the technical ability of the slide reader. High quality malaria microscopy has been difficult to implement outside reference laboratories. In order to improve quality of malaria microscopy in hospitals and health centers, the Senegal National Malaria Control Program (NMCP) adopted a strategy of giving district and select hospital laboratory technicians a week of intensive training followed by supervision visits during which slides were taken from each laboratory for quality control. In 2009, 42 technicians were trained, with average pre-test and post-test scores of 55% and 64%, respectively. With expert microscopist reading as the gold standard, of 596 slides selected for quality control, trainees had a sensitivity of 95% and specificity of 73%, with 6.5% of slides unreadable. In 2011, 53 technicians were trained, with average pre-test and post test scores of 75% and 87%, respectively. Among 866 slides selected for quality control, sensitivity was 95% and specificity was 77%, with 1.8% of slides unreadable. While laboratory technicians read slides with a high degree of sensitivity and reasonable specificity, the quality of slide preparation and coloration was noted to be mediocre, and very few technicians performed speciation or parasite density. To further improve quality of microscopy at hospitals and health centers, in 2013 regional biologists will be trained to help with supervision, the technician from each of the 76 district health centers will be retrained, all hospital and district health center labs will receive onsite supervision including proficiency testing from a standardized panel, and slide quality control. The microscopy quality control of the Senegal NMCP follows the recommendations of the World Health Organization in health facilities nationwide for parasite detection, speciation, and quantification of *parasitemia*. Improving quality of blood film microscopy at the peripheral level is important to improving diagnosis of low level *parasitemia* in the setting of decreasing transmission.

799

CORRELATING QUANTITATIVE REAL-TIME PCR TO RAPID ANTIGEN DETECTION ASSAY AND QUALITATIVE PCR RESULTS IN ISOLATED *PLASMODIUM FALCIPARUM GAMETOCYTEMIA*

Rachel Lau[1], Filip Ralevski[1], **Andrea K. Boggild**[2]

[1]Public Health Ontario Laboratories, Toronto, ON, Canada, [2]University of Toronto, Toronto, ON, Canada

Microscopic examination of stained thick and thin blood smears is the gold standard method for laboratory diagnosis of malaria, and can differentiate clinically relevant asexual *parasitemia* from clinically irrelevant isolated *gametocytemia*. Microscopy is time consuming, labour intensive, and requires significant technical expertise to perform. Rapid antigen detection assays are less labour intensive, but still may not reliably differentiate isolated *gametocytemia* from asexual *parasitemia*, which is important in a non-endemic laboratory context. To determine if Ct values on *Plasmodium* genus and *P. falciparum*-specific PCR assays targeting the 18S rRNA gene correlate to positivity of rapid antigen detection assay (Binax NOW Malaria), and 18S rRNA gene copy number, we analyzed samples from Ontario patients with isolated *P. falciparum gametocytemia*. Thirty-one samples containing microscopically-confirmed and isolated *P. falciparum* gametocytes, and no asexual stages, were identified and analyzed. Of these, 23 (74%) were follow-up specimens from patients known to have prior samples positive for asexual stages of *P. falciparum*, and 3 (10%) were from patients previously known to have mixed asexual stages of *P. falciparum* and *P. vivax*. Twenty-nine of 31 (93.5%) samples with isolated *gametocytemia* were positive for *Plasmodium falciparum*-specific histidine rich protein-2 (HRP-2) by rapid antigen detection assay. Nine of 31 samples (29.0%) were positive for *Plasmodium* genus aldolase by rapid antigen detection assay. Positivity of the aldolase band on rapid antigen detection assay was significantly correlated to lower mean *Plasmodium* genus ($p=0.002$) and *P. falciparum*-specific Ct values ($p<0.001$) by qualitative real time PCR. In addition, positivity of the aldolase band on rapid antigen detection assay was significantly correlated to higher mean *P. falciparum* 18S rRNA gene copy by quantitative real time PCR ($p=0.001$). These findings underscore that rapid antigen detection assays and conventional PCR assays do not reliably distinguish sexual from asexual *parasitemia* in a laboratory setting where clinical information may be unavailable. In addition, that rapid antigen detection assays can detect isolated *gametocytemia* has public health relevance in malaria endemic areas. Simple and rapid tests that can differentiate asexual from isolated sexual *parasitemia* are needed.

800

THE APPLICATIONS OF TRANSCRANIAL DOPPLER FOR THE ASSESSMENT OF INCREASED BRAIN VOLUME IN RETINOPATHY POSITIVE CHILDREN WITH CEREBRAL MALARIA

Robert B. Hamilton[1], Montfort B. Gushu[2], Cowles Chilingulo[2], Samuel D. Kampondeni[2], Karl B. Seydel[3], Xiao Hu[1], Terrie E. Taylor[3]

[1]University of California Los Angeles, Los Angeles, CA, United States, [2]Blantyre Malaria Project, Blantyre, Malawi, [3]Michigan State University, East Lansing, MI, United States

Having established a strong association between death and markedly increased brain volume (BV) per magnetic resonance imaging (MRI) in children with cerebral malaria (CM), we are now evaluating potential etiologies and surrogate measures of increased BV. Seven children with retinopathy-positive CM were evaluated with serial MRI studies and serial Transcanial Doppler (TCD) measurements. Patients were categorized on the basis of BV estimates (per MRI), and three TCD-derived values: mean cerebral blood flow ve*loci*ty (mCBFV), pulsatility index (PI, [systolic -diastolic]/mean ve*loci*ty) and Lindegaard ratio (mCBFV MCA/mCBFV ICA) into three categories: Increased BV with raised intracranial pressure, hyperemia, and "Hemodynamically Stable". The patients with increased BV and raised intracranial pressure were characterized by a clinically significant decrease in mCBFV coupled with an increase in PI which was accompanied by an increase in BV per MRI. In contrast, the hyperemia group showed an increased mCBFV and relatively low Lindegaard ratio during increased BV per MRI. Those deemed 'hemodynamically stable' remained unchanged for both the TCD and MRI measurements. Our findings demonstrate that repeated TCDs may provide information about BV, as confirmed by MRI, and may illuminate potential etiologies for increased BV in African children with CM. The point-of-care monitoring of TCD would allow clinicians to modify treatments at the bedside without the need of an MRI.

801

EXTENT AND DETERMINANTS OF MALARIA DIAGNOSTIC TEST USE AMONG FEBRILE CHILDREN UNDER FIVE IN THIRTEEN SUB-SAHARAN AFRICAN COUNTRIES USING STANDARDIZED NATIONAL SURVEYS

Emily W. Johansson[1], Peter W. Gething[2], Helena Hildenwall[3], Bonnie Mappin[2], Max Petzold[4], Stefan S. Peterson[1], Katarina E. Selling[1]

[1]Uppsala University, Uppsala, Sweden, [2]Oxford University, Oxford, United Kingdom, [3]Karolinska Institutet, Stockholm, Sweden, [4]Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

In 2010, the World Health Organization revised its malaria treatment guidelines to recommend diagnostic testing of suspected malaria cases before starting treatment. New data on malaria testing are available from national population-based surveys to examine the extent and determinants of malaria diagnostic test use among febrile children under five at the outset of this recommendation. We reviewed DHS, MICS and MIS in sub-Saharan Africa for inclusion of the main outcome: caregivers' reports that a child had fever in the previous 2 weeks and a finger/heel stick for testing. Surveys also needed to report main predictors: care sought for fever by facility type/level and malaria transmission intensity linked to datasets through geocoded survey clusters. 13 datasets met these inclusion criteria. We estimated total febrile children under five tested in 2010 in studied countries based on previously published methods. We examined factors associated with test uptake across pooled datasets using a multilevel logistic regression model with individuals nested within clusters and country as a random coefficient. Odds ratios for main predictors were adjusted for socioeconomic factors. Preliminary results indicate 4,896 (17%) febrile children under five were tested across studied countries. Compared to hospitals, the odds of a febrile child getting tested decreased by 37% if attending a non-hospital facility and by 68% if visiting a community health worker. Compared to no malaria risk settings, febrile children in low or moderate stable transmission areas were twice as likely to be tested. Febrile children with poorly educated mothers or living in poorest households were tested significantly less often than counterparts. Future analyses will examine differences in test uptake associated with attending public/private facilities as well as stratification of results for rural and urban contexts. Findings to date suggest significant inequities in malaria testing. An important step to close this gap is to prioritize test roll out to lower levels of care. This could improve fever management quality and more rational drug use where most non-complicated pediatric fevers are managed, particularly among poorest children.

802

IS THIS EVIDENCE OF SUCCESS IN MALARIA PREVENTION AND CONTROL MEASURES? IMPACT OF PREVENTION AND CONTROL MEASURES IN NIGERIA

Omotayo O. Ebong[1], Chijioke A. Nwauche[1], Kenneth C. Anugweje[2], Chinwe T. Ezirim[2], Ijeoma H. Ogbuehi[1], Lucy E. Yaguo-Ide[3], **Ifeyinwa N. Chijioke-Nwauche**[4]

[1]Centre for Malaria Research and Phytomedicine, University of Port Harcourt, Port Harcourt, Nigeria, [2]Lulu Briggs Health Centre, University of Port Harcourt, Port Harcourt, Nigeria, [3]Department of Paediatrics, University of Port Harcourt, Port Harcourt, Nigeria, [4]Department of Immunology and Infection, London School of Hygiene & Tropical Medicine, London, United Kingdom

Malaria is a preventable and treatable infectious disease which is a major public health issue in sub-Saharan Africa. Nigeria bears up to 25% of the malarial disease burden in Africa, hence contributing significantly to the one million lives lost per year in the region. The financial loss due to malaria is estimated to be about 132 billion Naira (8.8million US dollars). Efforts by the Federal Government and other stakeholders in the areas of mass distribution of insecticide treated nets (ITNs), community awareness programs, promotion of Artemisinin-based combination therapies (ACTs) and biolarviciding in order to achieve the sixth Millennium Development Goal of combating malaria have contributed to reduction in malaria incidence from 60 % to 43 % in Rivers State, Nigeria. There is need to provide evidence-based data to extrapolate the effect of these interventions on the overall burden of malaria in Nigeria. This study investigates malaria prevalence amongst asymptomatic subjects presenting for routine medical examination at the Lulu Briggs Health Centre at the University of Port Harcourt, Port Harcourt, Rivers State, Nigeria. Finger-prick blood samples were collected from 354 subjects, and were tested for parasitaemia using standard Rapid Diagnostic Test Kits (RDT; SD and Abbon), genotype, blood group and packed cell volume. Of the 354 samples tested, 39 (11%) tested positive for *Plasmodium* parasite. Among these, 32 (82.1%) were of AA, 6 (15.4%) AS, and 1 (2.5%) SSgenotype. Of the 39 samples positive for *Plasmodium* parasite, 23 (59%) were of O+, 10 (25%) of A+, 3 (8%) of AB+, and 3(8%) of the B+ blood groups. These results show an 11% prevalence of malaria among subjects studied. The results suggest that malaria control and prevention measures are having a degree of success in the country. This success may be the result of the aggressive campaigns to scale up malaria control measures, especially, prevention tools, like ITNs. These gains are impressive but must be sustained to avert a possible resurgence in the wake of ACT-resistance.

803

MICROENCAPSULATION INCREASES THE ANTIMALARIAL EFFICACY OF *PAVETTA CRASSIPES*

Martins Emeje[1], Ifeoma Obidike[1], Nneka Ibekwe[1], Dhiman Sarkar[2]

[1]National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria, [2]National Chemical Laboratory, Pune, India

The aim of this work was to develop *Pavetta* (PV)-loaded microcapsules and evaluate its antimalarial efficacy *in vitro*. Microcapsule dosage form with sodium alginate containing *Pavetta crassipes* alkaloidal extract was prepared using ionotropic gelation technology. The efficacy of the *pavetta*-loaded microcapsules was evaluated in chloroquine-sensitive (HB3) and chloroquine-resistant (FCM29) *Plasmodium falciparum* strains. Three standard drugs; artemisinin, chloroquine and quinine were used as reference standards for comparism. *Pavetta*-loaded microcapsules presented an adequate particle size (173 nm), narrow particle distribution (0.20), positive zeta potential (14mV) and high drug content (98.88 %) and encapsulation efficiency (67.01 %). The extract exhibited significant ($P < 0.05$) antiplasmodial effect against both *Pf* HB3 and *Pf* FcM29. The efficacy of *pavetta*-loaded microcapsules as measured by *in vitro* assay doubled (11.20) when the extract was microencapsulated compared with the free extract (22.08). IC_{50} of 11.20 and 10.60 µg/ml for *Pf* HB3 and *Pf* FcM29 respectively, representing an almost 50 % increased activity were recorded for *Pf* HB3 and *Pf* FcM29 respectively, compared with the IC_{50} of 22.08 and 21.91 µg/ml respectively for the free extract. Therefore, microencapsulation increased the interaction between the extract and the erythrocyte and this mechanism may be responsible for the extract's increased efficacy when microencapsulated. Our current findings show that PV and its microcapsule formulation may be a useful preparation in the treatment of acute chloroquine-sensitive and chloroquine resistant malaria.

804

ANTIPARASITIC ACTIVITY OF LACTOFERRIN PROTEIN ON *PLASMODIUM FALCIPARUM* AND LACTOFERRIN BOUND TO CHITOSAN NANOPARTICLES ON *P. BERGHEI* INFECTED MICE: AN *IN VITRO* AND *IN VIVO* STUDY

Namrata Anand

Postgraduate Institute of Medical Education and Research, Chandigarh, India

Lactoferrin protein is known as antimicrobial, antiparasitic, antitumor and immunomodulating in nature. The protein has shown its effect against

many parasites like *Giardia, Entamoeba.* But until now there is no study which has shown its inhibitory effect on malarial parasites *in vitro* as well as *in vivo*. Different concentrations of apo and Fe saturated lactoferrin at 40 and 80ug/ml were incubated with infected RBC's at initial *parasitemia* of 1%. After 24 and 48 hrs, the giemsa smear of the treated and non treated group were made and observed microscopically. ROS production and the *parasitemia* mean in all above groups were observed by flow cytometry (FACS). Also presence of ring, trophozoite and schizont was observed by confocal microscopy in treated as well as untreated groups. Study was divided in 2 groups of Balb/c mice labelled as: Infected mice with lactoferrin loaded Nanoparticle diet (given orally), infected mice fed on normal diet. Mice were inoculated with $1x10^6$ parasitized RBC's through I/P route. Following parameters were studied in all infected and uninfected groups at day 3, 6, 9 ,12 post infection: *parasitemia* in tail vein blood, spleen and liver weight and survival rate. The *parasitemia* count and ROS prodution was found to be decresed was found to be significantly decresed in Fe saturated lactoferrin protein group and apo protein group at 40 and 80 mg/ml concentration at 24 and 48 hrs post infection when compared with untreated group ($p<.005$). The malaria disease was developed in only 40% of the infected mice. At day 9 the *parasitemia* in case of normal diet mice was found to be >50% as compared to nanoparticles diet mice which showed only 10-15% *parasitemia* ($p<.005$). While mice with normal diet started dying on day 9 post infection. The mice given these nanoparticles orally resulted in survival upto 25 days. Two mice died at day 9 in case of normal diet group. The mice having normal diet showed enlargement of spleen and liver due to parasite sequestration as compared to mice which were on nanoparticles diet. ($p<-005$). In conclusion, the present study has shown good inhibitory effect of lactoferrin protein on intracellular parasites. Lactoferrin can be used as a theraputic drug for malarial infection.

805

THE *IN VIVO* ANTI-PLASMODIAL ACTIVITY OF *NUCLEALA LIFOLIA* ELIMINATES CEREBRAL AND HEPATIC PARASITEMIA AND REDUCES DAMAGE TO THE TISSUES' MICROSTRUCTURES

Innocent Onyesom

Delta State University, Abraka, Nigeria., Abraka, Nigeria

The high rate of malaria transmission and prevalence have been reported in the Niger-Delta wetland region of Nigeria. The inhabitants of this region rely heavily on local medicinal plants to treat malarial infection. One of such plant is *Nuclea latifolia* known to contain monoterpeneindol alkaloids. In this study, the *in vivo* antiplasmodial activity of *N. latifolia* and its potency to eliminate hepatic and cerebral malarial parasites, and abate oxidative tissue damage were investigated using mice model. Results show that the two doses (200 and 300mg/kg) of*N. latifolia*leaf extract significantly suppressed blood parasitaemia by 63.32% and 81.39%, respectively. These values compare well with the standard choloquine treatments whose chemosuppression was 90.39%. The leaf extract and chloroquine treatments eliminated hepatic and cerebral parasites, and abated the *Plasmodium berghei*-induced oxidative damage as indicated by the histopathological microstructures. *N. latifolia* holds promise in the treatment of malaria. Therefore, the bioactive ingredient should be structured and its mechanism of action deciphered in order to further understand the antiplasmodial activity of *N. latifolia.*

806

CARDIAC SAFETY OF MONTHLY DIHYDROARTEMISININ-PIPERAQUINE FOR MALARIA PROPHYLAXIS: A TWO ARM, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED COHORT STUDY

David Saunders[1], Chanthap Lon[1], **Pattaraporn Vanachayangkul**[1], Michele Spring[1], Mary So[2], Darapiseth Sea[3], Youry Se[1], Sok Somethy[2], Sut-Thang Phann[4], Soklyda Chann[1], Sabaithip Sriwichai[1], Nillawan Buathong[1], Worachet Kuntawunginn[1], Mashamon Mitprasat[1], Raveewan Siripokasupkul[1], Paktiya Teja-lisavadharm[1], Eugene Soh[5], Jessica Manning[1], Ans Timmermans[1], Charlotte Lanteri[1], Dounglas Tang[6], Jaranit Kaewkungwal[7], Char Meng Chour[3], Satharath Prom[3], Mark Haigney[5], Louis Cantilena[5]

[1]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]Royal Cambodian Armed Forces, Phnom Penh, Cambodia, [3]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia, [4]National Center for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia, [5]F. Edward Hebert School of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [6]Fast Track Biologics, Bethesda, MD, United States, [7]Centers for Excellence in Bioinformatics (BIOPHICS), Mahidol University, Bangkok, Thailand

Dihydroartemisinin-piperaquine (DP), the current first line recommended treatment of uncomplicated *Plasmodium falciparum* and *P. vivax* in Cambodia, and worldwide has previously been shown to be of benefit as a monthly medication to prevent malaria. We evaluated the safety and efficacy of monthly DHA-piperaquine in a compressed 2 day dosing regimen. Protective efficacy and cardiac safety of a monthly course of Dihydroartemisinin-piperaquine were compared in a two arm, randomized, double-blind, placebo-controlled cohort study with 2:1 treatment allocation. Healthy volunteers in high risk areas along the Thai-Cambodian border were administered 180 mg DHA and 1440 mg PIP as combination tablets for 2 days once per month within 1-3 hours of a meal. Volunteers were followed for 4 months with electrocardiographic assessment of the QTc interval and piperaquine drugs levels at 0, 4, 24 and 28 hours post dosing. The study, with a planned enrollment of 231 volunteers was suspended after only 6 weeks (69 volunteers enrolled) when 4 volunteers met a pre-specified cardiac safety endpoint with >500 ms QTcF prolongation. Effect peaked at approximately 4 hours after piperaquine dosing, and lasted 4-8 hours. Mean QTcF prolongation of 50ms over placebo was seen at Tmax on day 2. The study was halted after review by an independent data safety monitoring board. Given the utility of DHA-piperaquine as one of the few remaining effective antimalarials in Cambodia, until the clinical significance of the findings can be more thoroughly evaluated, compressed 2 day treatment courses are best avoided. Repolarization risk using conventional 3 day doses of DHA-piperaquine can be mitigated by fasting for 3 hours before/after administration. Avoiding repeated dosing, unintentional overdose, and coadministration of other QT prolonging medications is also recommended. Other potential safety interventions including EKG and electrolyte monitoring are rarely available in settings where malaria is endemic.

807

EXPLORING PHARMACOKINETICALLY GUIDED POTENTIAL ANTIMALARIAL COMBINATION THERAPY

Wahajuddin Wahajuddin, Kanumuri S. Raju, Isha Taneja, Shio K. Singh

CSIR-Central Drug Research Institute, Lucnkow, India

Lumefantrine (LUME) in combination with artemether is the current first-line therapy for treatment of uncomplicated *falciparum* malaria. All the currently recommended firstline antimalarial combinations include an artemesinin derivative as one of the therapeutic agent. The commercial

availability of artemisinins is scarce due to their natural biodistribution as well as tedious and costly extraction procedure. Also, resistance has been observed for even artemisinins now. There are few cases reported for development of resistance to present combinations. Clinical treatment failure has even been reported for the firstline combination artemether-lumefantrine. 99-411 and 97-78 are short acting candidate antimalarials presently under clinical trials in India. Thus, we assessed the *in vivo* pharmacokinetic compatibility of trioxane candidate antimalarial, 99-411 and 97-78 with longer half-life partner drug, LUME and explored their potential to be developed as an effective antimalarial combination treatment. The examination of 99-411 and 97-78 with LUME as futuristic antimalarial combination indicates that LUME co-administration significantly enhanced the exposure of 99-411, which may be beneficial for the therapeutic efficacy. However, LUME exposure reduced significantly when co-administered with 97-78 which was not significantly affected. LUME did increase the AUC, C_{max}, Tmax of co-administered 99-411, however, the plasma half-life was found to decrease. On exploring the mechanisms that may be responsible in this additive interaction, the intestinal permeability studies show that LUME significantly increased the effective permeability of 99-411 by almost 2 fold while no such effect of 99-411 on LUME was seen. The pharmacokinetic evaluation of the two combinations suggests that LUME with 99-411 seems to have therapeutic merit and it may be useful in delaying the development of resistance against both the drugs which is the major reason of antimalarial therapy failure world over.

808

THE P-TYPE CATION-TRANSPORTER ATPASE, PFATP4, IS A MULTIDRUG RESISTANCE ASSOCIATED GENE IN *PLASMODIUM FALCIPARUM*

Erika L. Flannery[1], Case W. McNamara[2], Fengwu Li[1], Kerstin Gagaring[2], Micah Manary[1], Elizabeth A. Winzeler[1]

[1]University of California San Diego, San Diego, CA, United States, [2]The Genomics Institute of the Novartis Research Foundation, San Diego, CA, United States

Efforts to control malaria are continuously challenged by the emergence of drug resistance. New drugs will thus be needed for treatment and control efforts aimed at eliminating this disease that causes 700,000 unnecessary deaths annually. Aminopyrazoles are a unique class of antimalarial compounds with potent activity against *Plasmodium falciparum* blood stage parasites which were identified in a cellular antiparasitic screen and whose mechanism of action is unknown. To investigate their mechanism of action we pressured parasites until they acquired low-level resistance to a representative member of the aminopyrazole series, GNF358, creating three independent resistant lines. The IC50 value of each clone was 3-10 fold greater than that of the parental Dd2 clone (148 nM). Whole-genome sequencing of the resistant lines showed that each had acquired independent mutations in a P-type cation-transporter ATPase, PfATP4 (PF3D7_1211900), a gene implicated in resistance to another, structurally unrelated, class of antimalarials, the spiroindolones which are currently in Phase II clinical trials. Not unexpectedly, GNF358-resistant lines also exhibited selective cross-resistance to the spiroindolones with IC50 values shifted more than 5-fold greater than that observed for the drug-sensitive parental line. Like the spiroindolones, we show that GNF358 inhibits gametocyte transmission as well as disrupts sodium homeostasis in the parasites. Additionally, transgenic parasite lines harboring directed mutations in PfATP4 also show cross-resistance with GNF358. Our data show PfATP4 plays an important role in cellular processes, can be inhibited by two distinct antimalarial pharmacophores and supports the recent observations that PfATP4 is a critical drug target.

809

SAFETY AND EFFICACY OF REPEATED ADMINISTRATION OF PYRONARIDINE-ARTESUNATE OR DIHYDROARTEMISININ-PIPERAQUINE VS ARTESUNATE-AMODIAQUINE OR ARTEMETHER-LUMEFANTHRINE OVER A TWO-YEAR PERIOD IN CHILDREN AND ADULT PATIENTS WITH ACUTE UNCOMPLICATED *PLASMODIUM S.P.* MALARIA AT NIANGOLOKO SITE IN BURKINA FASO

Issiaka Soulama, Sodiomon B. Sirima

Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso

ACTs have been recommended as first line treatment for uncomplicated malaria by WHO. However, the safety of repeated administration of the same drug to an individual is not documented.The present study aims to assess the safety and effica.cy of repeated administration of pyronaridine-artesunate (PA) or dihydroartemisinin-piperaquine (DHA-PQ) vs artesunate-amodiaquine (ASAQ) or artemether-lumefanthrine (AL) over a period of 2 year. The primary endpoints are to assess the non-inferiority of PA or DHA-PQP vs ASAQ or AL in term of the incidence rate of uncomplicated malaria in children and adults treated with repeated ACT therapy over a 2 year observation period and to assess the non-inferiority of PA or DHA-PQP vs ASAQ or AL in term of PCR corrected and uncorrected ACPR at D28 and D42 (WHO definitions 2009).We are carrying out a comparative, randomized, open label longitudinal clinical trial involving children and adults with uncomplicated *Plasmodium sp.* malaria. In the current study, at the enrolment, eligible participants are randomly assigned to one of the treatment arm to receive 3-day course of either DHA-PQ or ASAQ. They are then followed up for 42 days. Standard assessments for antimalarial efficacy and safety trials are conducted. Hematology, biochemistry, PK and 12 Lead ECG are done at various predefined timepoints. During their subsequent episodes, participants receive the same drug and will go through the same trial procedures as for the initial episode. The partial results from the first year of the follow up with DHA and ASAQ arms showed that 30 of 85 patients in the ASAQ arm compared to 20 of 85 in the DHA arm experienced 2 malaria episodes. The Adequate clinical and parasitological response (ACPR) by day 28 was 91.55 % in ASAQ arm compared to 100% in DHA-PQ arm. The total treatment failures with ASAQ by day 28 (not PCR corrected) were 8.45 % whilst failures in DHA-PQ arm were 0%. The 42 cure rates (not adjusted by PCR) were 69.88 % and 96.35 % in ASAQ and DHA-PQ arms respectively. Our preliminary results confirmed the two drugs are safe and indicated that in high transmission region the antimalarial efficacy of DHA-PQ was superior to that ASAQ. The study is ongoing to complete the entire sample size and follow the patients over a period of two years.

810

INTRANASAL ADMINISTRATION OF ARTESUNATE AND ERYTHROPOIETIN TO TREAT CEREBRAL MALARIA

Stephane Picot, Anne Marijon, Laetitia Taurines, Guillaume Bonnot, Adeline Lavoignat, Gaetan Herbet, Anne-Lise Bienvenu

Malaria Research Unit, Lyon, France

Despite treatment with anti-malarial drugs, patients die from cerebral malaria and too many are discharged with neurological deficits. In the long term, 1/4 of survivors have neurological and cognitive impairments years after exposure. Currently, treatment of malaria is based mainly on drugs such as artesunate. Erythropoietin antagonizes both the production and the effects of pro-inflammatory cytokines and limits perfusion-reperfusion injury in a variety of tissues. Systemic administration of recombinant human EPO (rhEPO) is protective in animal models for stroke, spinal cord injury, myocardial infarction, and ischemia-reperfusion among many others. Human trials with rhEPO have demonstrated tissue protective activities in stroke, multiple sclerosis, multi-system trauma requiring intensive care support similar to those observed in the preclinical models. We first reported the protective effect of rhEPO in murine cerebral malaria.

We also demonstrated that high doses Erythropoietin were safe for children suffering severe cerebral malaria. Use as adjunctive treatment associated with anti-malarial drug, Epo could help to de-crease the early mortality rate of severe malaria. Experimental malaria was induced in mice infected with *Plasmodium berghei* ANKA. Animal experiments were conducted in agreement with ethical and general rules for animal protection. Drugs were used at serial dilutions and administered either I.P. (control groups), using in-tranasal spray device, or dropwise after anesthesia. Mice were monitored for signs of CM, including body weight, hematocrit, ataxia, paralysis, deviation of the head, convulsions, and coma. Parasitemia were determined using Giemsa-stained thin blood films. Intranasal administration of artesunate was highly efficient to decrease blood *parasitemia* in *P. berghei* infected mice. The cure rate using 40mg/kg b.w; was 100% for two successive experiments (60 mice). Same efficacy was obtained when the artesunate dose was deacreased. This is the first evidence that artesunate and erythropoietin treatment could be administered via intranasal route.

811

THE MECHANISMS OF ACTION OF TWO NEW BENZOXABOROLES AGAINST *PLASMODIUM FALCIPARUM*

Ebere Sonoiki[1], Denghui Guo[2], Caroline Ng[3], Vida Ahyong[4], Sumit Rathore[2], Jiri Gut[2], Jenny Legac[2], M.r.k. Alley[5], Yvonne R. Freund[5], Chen Dong[5], Eric Easom[5], Jacob Plattner[5], Virginia Sanders[5], Yong-Kang Zhang[5], Fernando Rock[5], David Fidock[3], Joseph L. DeRisi[4], Philip J. Rosenthal[2]

[1]Division of Infectious Diseases and Immunology, School of Public Health, University of California, Berkeley, CA, United States, [2]Department of Medicine, University of California, San Francisco, CA, United States, [3]Department of Microbiology and Immunology and Medicine, Columbia University, New York, NY, United States, [4]Department of Biochemistry and Biophysics, University of California, San Francisco, CA, United States, [5]Anacor Pharmaceuticals, Palo Alto, CA, United States

There is an urgent need for new antimalarials, preferably with novel mechanisms of action. We have identified several boron-containing compounds with excellent *in vitro* potency and *in vivo* efficacy (AN3661: IC_{50} 37 nM against W2-strain *Plasmodium falciparum*, ED_{90} 0.3 mg/kg against murine *P. berghei*; AN6426: IC_{50} 196 nM, ED_{90} 7.44 mg/kg). AN3661, AN6426, and related compounds were tested for stage-specificity by incubation with test compounds for 8 h intervals across the parasite erythrocytic life cycle. They were also tested for inhibition of protein synthesis by comparing the incorporation of 14[C]Leu by parasites treated with test compounds or controls and for inhibition of plasmodial leucyl tRNA synthetase (LeuRS), an antimicrobial target of other benzoxaboroles, by monitoring 14[C]Leu incorporation in cytoplasmic extracts including exogenous tRNA. The trophozoite stage was most sensitive to AN3661 and AN6426. Dose-dependent inhibition of both protein synthesis and LeuRS activity was observed for AN6426, but not AN3661 or the control artemisinin, supporting different mechanisms for the different benzoxaboroles. To gain further insight into mechanisms of action we selected for *P. falciparum* with decreased sensitivity to AN3661 or AN6426 by culturing W2-strain parasites in step-wise increasing concentrations of the compounds and Dd2-strain parasites in one high concentration of AN3661. Cross-resistance was not seen between parasites selected with AN3661 and those selected with AN6426. Whole genome sequencing of multiple clones selected for resistance to AN3661 revealed several SNPs in a gene that codes for homologs of mammalian cleavage and polyadenylation specificity factor (CPSF; PF14_0364). Sequencing of parasites selected for resistance to AN6426 showed several SNPs in the editing domain of the predicted cytoplasmic LeuRS gene (PFF1095w). In summary, we offer strong evidence for unique antimalarial mechanisms of action for two benzoxaboroles, identifying two novel antimalarial drug targets. Further investigation of these novel benzoxaborole mechanisms is underway.

813

EFFICACY, SAFETY AND POPULATION-PHARMACOKINETICS OF THE ARTESUNATE-MEFLOQUINE (ASMQ) FIXED DOSE COMBINATION VERSUS ARTEMETHER-LUMEFANTRINE FOR THE TREATMENT OF UNCOMPLICATED *FALCIPARUM* MALARIA IN AFRICAN CHILDREN

Monia Guidi[1], Laurent A. Decosterd[1], Thomas Mercier[1], Chantal Csajka[1], Bernhards Ogutu[2], Gwénaëlle Carn[3], **Jean-René Kiechel**[3]

[1]Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland, [2]Kenya Medical Research Institute, Kisumu, Kenya, [3]Drugs for Neglected Diseases initiative, Geneva, Switzerland

WHO guidelines recommend fixed-dose combinations of artemisin-based combination therapies (ACTs) to treat uncomplicated *Plasmodium falciparum* malaria. The use of the combination of AS with MQ (non-fixed and fixed) is well documented in Asia (high efficacy, good safety). In Africa, however, there is limited experience with the combination and no data regarding MQ pharmacokinetics in children treated with the fixed dose combination (FDC). Our objective was to evaluate the efficacy, safety and population-pharmacokinetics of fixed dose ASMQ in children. A randomized clinical study was conducted in children under 5 years of age in Kenya, Tanzania and Burkina Faso. It consists of a 3-day treatment period and 60-day follow-up. Patients received either ASMQ FDC or Artemether-Lumefantrine. The randomized clinical study is still ongoing. MQ data were obtained in 50 Kenyan patients and analysed using population pharmacokinetics (NONMEM®). Blood samples were collected before dosing at day (D) 0 and at 5 other time points on D0, D2, D3, D7 and 1 sample at randomly selected days during the follow-up visits (days 28 35 42, 49, 56 or 63). A two-compartment model best described MQ pharmacokinetics. The estimates and the variability (CV%) of the pharmacokinetic parameters were a systemic clearance of 0.20 L/h (40%), volumes of distribution of the central and peripheral compartment of 43.5 L (51%) and 24.2 L, an inter-compartmental clearance of 0.12 L/h, and an absorption rate constant of 0.17 h-1 (97%). No available covariates could explain the large inter-patient variability observed in the pharmacokinetic parameters, except for age on Ka. Average absorption time was 6.3 h (range: 1.4-27.4 h) and mean terminal half-life 14.1 days (9.3-46.5 days). MQ pharmacokinetics present large inter-patient variability in children treated with fixed dose regimen. Clearance and volume of distribution of MQ in children is lower than in adult patients, but the terminal elimination half-life and mean absorption time are of similar magnitude.

814

RELATIVE BIOAVAILABILITY OF CO-ADMINISTERED FORMULATIONS OF AZITHROMYCIN (AZ) MICROSPHERE AND CHLOROQUINE (CQ) TEST FORMULATION COMPARED WITH CO-ADMINISTERED IMMEDIATE RELEASE INDIVIDUAL AZ AND CQ TABLETS IN HEALTHY ADULT SUBJECTS

Qinying Zhao, Rong Wang, Melissa O'Gorman, Penelope Crownover, Richa Chandra

Pfizer Inc., Groton, CT, United States

The combination of AZ and CQ has shown synergistic activity against *Plasmodium falciparum in vivo* and *in vitro*. The better gastrointestinal (GI) tolerability of azithromycin (AZ) extended-release (ER) microsphere formulation (Zmax®) relative to AZ immediate-release (IR) formulation allows administration of a higher single dose of AZ with acceptable GI tolerability. CQ phosphate was formulated as an exploratory microsphere formulation to mask the bitter taste of CQ that could be co-administered with AZ ER for malaria treatment or prophylaxis. This Phase 1, open-label, single-dose, parallel-group study evaluated the relative bioavailability of co-administered AZ ER (2 g) and experimental microsphere formulation of CQ (620 mg base; Test Formulation) compared with co-administered IR individual commercial tablets of AZ (2 g) and CQ (600 mg CQ base; Reference Formulation) in 40 healthy adults. Subjects were confined to the Clinical Research Unit for 2 days with additional clinic visits on Days

3-5 following enrollment. Blood samples were drawn at pre-specified times up to 96 hours post dosing and analyzed to determine AZ and CQ concentrations. Noncompartmental analysis of concentration-time data was used to calculate pharmacokinetic parameters of maximal observed drug concentration (Cmax), time to Cmax (Tmax), and area under the drug concentration-time curve from time zero to the time of the last quantifiable concentration (AUClast). ANOVA was used to compare natural log-transformed AUClast and Cmax with group (main effect) and log weight (covariate). Safety evaluations included monitoring of adverse events (AE), clinical laboratory tests, and vital signs. All enrolled subjects completed the study. The relative bioavailability AUClast values (90% CI) of serum AZ and plasma CQ (Test Formulation) were 73.79% (63.99%-85.08%) and 105.47% (93.39%-119.11%), respectively, compared to the Reference Formulation. The AE profile and GI tolerability were better for the Test formulation compared to the Reference Formulation. No safety issues were identified in either treatment.

815

TEACHING AN OLD DRUG NEW TRICKS: NOVEL AMODIAQUINE ANALOGUES WITH POTENT ANTIPLASMODIAL ACTIVITY AND A POTENTIALLY IMPROVED SAFETY PROFILE

Dennis S. Ongarora[1], Collen M. Masimirembwa[2], Kelly Chibale[1]

[1]University of Cape Town, Cape Town, South Africa, [2]African Institute of Science and Biomedical Technology, Harare, Zimbabwe

The devastating morbidity and high mortality associated with malaria infection, coupled with the development of drug resistance to new treatment regimens almost as soon as they are introduced, underline the imperative for sustained research into new antimalarial agents. It is important that these new agents are not only efficacious but also safe. Drug discovery is a costly and time consuming process. One of the approaches adopted to circumvent or mitigate against this bottleneck is the use of existing drugs as lead compounds upon which suitable chemical modifications can be carried out. The 4-aminoquinoline antimalarial amodiaquine exhibits excellent activity against both sensitive and resistant *Plasmodium falciparum* strains. Its use is, however, restricted due to severe hepatotoxicity and agranulocytosis. This toxicity is attributed to metabolic activation to the quinone imine and aldehyde metabolites, respectively. These reactive metabolites bind to and disrupt the function of cellular macromolecules. We hypothesized that masking the functionality responsible for the formation of these reactive metabolites in the form of a benzoheterocyclic system would block bioactivation while retaining potent antiplasmodial activity against both drug sensitive and resistant strains of *P. falciparum*. As a proof of concept, novel benzoheterocyclic analogues were synthesized and subjected to glutathione trapping studies using electrochemical oxidation online with electrospray ionization mass spectroscopy (EC-ESI/MS) to give an indication of the potential for reactive metabolite formation. The results confirmed that the potential to form the quinone imine was eliminated but potent antiplasmodial activity was retained. In conclusion, these results demonstrate that benzoheterocyclic analogues based on amodiaquine are promising leads in the discovery of novel safer aminoquinoline antimalarials.

816

TRANSMISSION BLOCKING PROPERTIES OF ANTIMALARIALS

Martijn Timmerman[1], Robert Sauerwein[1], Didier Leroy[2], **Koen Dechering**[1]

[1]TropIQ Health Sciences, Nijmegen, Netherlands, [2]Medicines for Malaria Venture, Geneva, Switzerland

Malaria transmission is critically dependent on sexually differentiated gametocytes that develop in the bloodstream of the human host and are infectious to the mosquito vector. These gametocytes are not sensitive to the vast majority of marketed antimalarials and patients treated for clinical malaria may still carry gametocytes and transmit the disease. Successful elimination of malaria depends on the generation of new intervention tools that target the transmission stages of the parasite. We have evaluated the transmission-blocking potential of components of Artimisinin based Combination Therapy and of a number of candidate drugs from the Medicines for Malaria Venture R&D portfolio. To this end, we tested compound effects against *Plasmodium falciparum* gametocytes in a newly developed pLDH assay that monitors gametocyte metabolic activity. In addition, we developed an assay to quantify gametogenesis capacity and have modified the protocol for a Standard Membrane Feeding Assay (SMFA) in order to establish precise dose response measurements on sporogonic development in the mosquito. Our assays revealed distinct mechanisms of action for different compounds. Artemisinins, and in particular the active metabolite dihydroartemisinin, show activity against gametocytes, which results in a partial block in gamete formation and a full block in oocyst development. Quantitative measurements show that DHA is 30-fold more potent against asexual parasite stages than against oocyst formation in the mosquito. Other compounds, such as pyronaridine, do not affect gametocyte pLDH activity but block oocyst development, suggesting they act directly against the sporogonic stages in the mosquito midgut. Lastly, we tested a number of compounds from the MMV drug discovery portfolio. The majority of compounds in early clinical development exhibit multi-stage activity and block transmission. Our data indicate differential effects on oocyst intensity and oocyst prevalence (number of infected mosquitoes), which depend on the parasite exposure and specific mechanism of action. Our studies identified a small number of compounds for which the efficacy is relatively independent of the parasite exposure.

817

LEAD OPTIMIZATION OF BROAD-SPECTRUM ANTIMALARIAL ACRIDONES

Jane X. Kelly[1], Rosie Dodean[2], Yuexin Li[2], Brandon Pybus[3], Qigui Li[3], Sean Marcsisin[3], Jason Sousa[3], Isaac Forquer[1], Rolf Winter[1], Dave Hinrichs[1], Stephanie Huezo[4], Roland Cooper[4], Mike Riscoe[1], Victor Melendez[3]

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

We have previously reported the discovery of a novel antimalarial acridone chemotype that displays efficacy against sporozoite-induced *Plasmodium* infection in addition to efficacy against blood stage parasites. An aggressive optimization process not only expanded our chemical library but more importantly produced a new lead candidate with significantly improved efficacy, both *in vitro* and *in vivo*. The new lead candidate also exhibits potent activity against atovaquone-resistant parasites. Details of the design, chemistry, structure-activity relationships (SAR), safety, metabolic studies, and mechanism of action will be presented.

818

PHASE 2 RANDOMIZED PROOF OF CONCEPT STUDY EFFICACY TRIAL COMPARING AN INVESTIGATIONAL AMINOQUINOLINE ANTIMALARIAL (AQ-13) TO COARTEM IN MALIAN MALES WITH UNCOMPLICATED MALARIA

Ousmane A. Koita[1], Aliou Sissako[1], Lansana Sangare[1], Haiyan Deng[2], Trevor A. Thompson[2], Sahare Fongoro[1], Boubakar Diallo[1], Seydou O. Doumbia[1], Lamine Traore[1], Ayouba Diarra[1], Frances J. Mather[2], Asif Anwar[2], Joon Sup-Yeom[3], David M. Mushatt[2], Mark Sather[4], Donald J. Krogstad[2]

[1]University of Bamako, Bamako, Mali, [2]Tulane University Health Sciences Center, New Orleans, LA, United States, [3]Sungkyunkwan University, Seoul, Republic of Korea, [4]ScottCare, Cleveland, OH, United States

Although artemisinin-combination therapies (ACTs) are the recommended first-line treatment for uncomplicated *Plamodium falciparum* malaria, there

is increasing concern about artemisinin resistance because of prolonged parasite clearance times in Southeast Asia. For this reason, it would be extremely valuable to have alternatives to ACTs that were effective against chloroquine(CQ)-resistant *P. falciparum*, economical and could be given orally. Our previous studies have shown that aminoquinolines (AQs) with modified side chains such as AQ-13 are active against CQ- and multi-resistant *P. falciparum in vitro* and in a squirrel monkey model of human infection with CQ-resistant *P. falciparum*, are as safe as CQ in healthy human subjects and have similar pharmacokinetics. This study randomizes adult Malian males (≥18 years of age) with uncomplicated *P. falciparum* malaria to receive 1,750 mg of AQ-13 orally over 3 days or the currently recommended dose of Coartem (480 mg artemether + 2,880 mg lumefantrine) orally over 3 days. The major endpoint is treatment failure (failure to reduce asexual *parasitemia* to <25% of baseline by day 3, clear all asexual parasites by day 7 or recurrent infection with the same parasite genotype between days 8 and 42). Secondary endpoints include clinically apparent adverse events, parasite and fever clearance times, time to recurrent infection, pharmacokinetic parameters for AQ-13 and prolongation of the QT (QTc) interval. Tertiary endpoints include the pharmacokinetics of AQ-13 metabolites and the occurrence of pruritus in persons receiving AQ-13. Based on a start date in June, we expect that the initial 66 subjects will have been enrolled by October or earlier and that the initial results should be available for presentation in November.

819

DEVELOPMENT OF A HUMANIZED PSEUDO-LIVER NOD/SCID MOUSE MODEL OF DRUG METABOLISM

Kristina S. Wickham, Rosemary Rochford

State University of New York Upstate Medical University, Syracuse, NY, United States

Humanized mouse models are a powerful tool for direct investigation of preclinical drugs and human disease. We are currently developing a humanized SCID mouse model to assess the hemolytic potential of candidate antimalarial drugs in the context of glucose-6-phosphate dehydrogenase deficiency ($G6PD^-$), the most common human enzymopathy which results in hemolytic anemia in patients treated with drugs such as primaquine (PQ). We previously reported on the development and validation of a human (hu)RBC-SCID mouse model in which NOD/SCID mice are given daily transfusions of human red blood cells from $G6PD^-$ donors. Our efforts aim to test 8-aminoquinoline antimalarial drug candidates derived from the parent drug PQ for hemolytic toxicity. A major limitation of humanized mice is that they retain physiological characteristics of an animal system that are not identical to a human system such as cytochrome p450 (CYP)-dependent drug metabolism. It is therefore difficult to predict pathways of CYP-dependent metabolism based on our huRBC-SCID model because the variation in functionality of CYP family members between mice and humans is incompletely understood. The metabolic species responsible for toxicity in the 8AQ class and their associated pathways are also not fully understood. Ideally, humanized livers could be combined with the huRBC-SCID model to assess the role of human metabolic activity in the background of hemolytic toxicity induced by antimalarials. Since technical challenges limit this approach, we propose establishing s.c. tumors in NOD/SCID mice arising from the HepG2 human hepatoma cell line as a viable alternative. HepG2 cells retain some characteristics of a normal human liver and can be transfected with human CYP to examine the role of an individual CYP in PQ metabolism. To determine relative HepG2 CYP expression both *in vitro* and *in vivo* compared to normal liver, RT-qPCR was performed to analyze expression of CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 relative to β-actin in HepG2 cells, HepG2 tumors established in NOD/SCID mice and normal human liver. Results show that CYP expression is reduced in both HepG2 cells *in vitro* and *in vivo* compared to normal liver, demonstrating that this cell line may be a suitable vehicle for establishing a pseudo-liver in our $G6PD^-$ model. Ongoing studies are aimed at expressing CYP2D6 and CYP3A4 in pseudo-livers to evaluate their role in PQ metabolism.

820

SONTOCHIN AS A GUIDE FOR DEVELOPMENT OF CHLOROQUINE REPLACEMENT DRUGS

Sovitj Pou[1], Rolf Winter[1], Yuexin Li[1], Jane Xu Kelly[1], Isaac P. Forquer[1], J. Stone Doggett[2], David J. Hinrichs[3], **Michael K. Riscoe**[3]

[1]Portland Veterans Affairs Medical Center, Portland, OR, United States, [2]Oregon Health and Science University, Department of Medicine, Portland, OR, United States, [3]Oregon Health and Science University, Department of Molecular Microbiology and Immunology, Portland, OR, United States

Sontochin was the original chloroquine replacement drug, arising from research by Hans Andersag two years after chloroquine ("Resochin") had been abandoned due to the mistaken perception that it was too toxic for human use. We found that sontochin, i.e., 3-methyl-chloroquine, retains significant activity against chloroquine resistant strains of *Plasmodium falciparum in vitro*. As a result we began to explore sontochin analogs, "pharmachins", with alkyl or aryl substituents at the 3-position of the 4-aminoquinoline core. Modified with an aryl substituent in the 3-position of the 7-chloroquinoline ring, PH-203 exhibits low nanomolar IC_{50} values against drug sensitive and multidrug resistant strains and *in vivo* efficacy against patent infections of *P. yoelii* in mice that is superior to chloroquine. With sontochin and PH-203 as structural leads for optimization we have continued to elaborate a library of pharmachins varying the aryl substituent at the 3-position while optimizing the scaffold for *in vitro* activity and *in vivo* efficacy against malaria in a murine model of the disease. As part of the test cascade we characterized selected molecules for pharmacokinetics and for off-target effects including cytotoxicity and blockade of the human Herg channel. We have also profiled our frontrunners against a broad range of targets including human receptors, ion channels, transporters, enzymes and second messengers to guide the down-selection process. Our results show that novel 3-position aryl pharmachins represent potential chloroquine replacement drugs for treating malaria in humans.

821

OPTIMIZATION OF A LIGASE DETECTION REACTION-FLUORESCENT MICROSPHERE ASSAY FOR THE CHARACTERIZATION OF RESISTANCE-MEDIATING POLYMORPHISMS IN AFRICAN SAMPLES OF *PLASMODIUM FALCIPARUM*

Norbert P. LeClair[1], Melissa D. Conrad[1], Frederick N. Baliraine[1], Christian Nsanzabana[1], Samuel L. Nsobya[2], Philip J. Rosenthal[1]

[1]University of California San Francisco, San Francisco, CA, United States, [2]Makerere University, Kampala, Uganda

Genetic polymorphisms in the malaria parasite *Plasmodium falciparum* mediate alterations in sensitivity to important antimalarial drugs. Surveillance for these polymorphisms is helpful in assessing the prevalence of drug resistance and designing strategies for malaria control. Multiple methods are available for the assessment of *P. falciparum* genetic polymorphisms, but these suffer from low throughput, technical limitations, and high cost. We have optimized and tested a multiplex ligase detection reaction-fluorescent microsphere (LDR-FM) assay for the identification of important *P. falciparum* genetic polymorphisms. For 83 clinical samples from Kampala, Uganda, a region where transmission intensity and infection complexity are both high, DNA was extracted from dried blood spots, genes of interest were amplified by PCR, amplicons were subjected to multiplex ligase detection reactions to add bead-specific oligonucleotides and biotin, fragments were hybridized to magnetic beads, and polymorphism prevalences were assessed fluorometrically in a multiplex format. A total of 19 alleles from the pfcrt (codons 72-76 CVMNK, CVIET, or SVMNT), pfmdr1 (N86Y, Y184F, S1034C, N1042D, D1246Y), pfmrp1 (I876V), pfdhfr (N51I, C59R, S108N or T, I164L) and pfdhps (S436A, A437G, K540E, A581G, A613S) genes were analyzed by LDR-FM and restriction fragment length polymorphism (RFLP) analysis.

Considering samples with results from both assays, concordance between the assays was good, with 78-100% of results identical at individual alleles, most non-concordant results differing only between a mixed and pure genotype call, and full disagreement at individual alleles in only 0-3% of results. We estimate the LDR-FM assay to offer much higher throughput and lower cost compared to RFLP analysis. Our results suggest that the LDR-FM system offers an accurate high throughput means of classifying genetic polymorphisms in field samples of *P. falciparum*.

822

SAMPLING DESIGN FOR THE PARASITE CLEARANCE ESTIMATOR: TWO COMPLEMENTARY METHODOLOGICAL APPROACHES

Jennifer A. Flegg[1], Kris M. Jamsen[2], Philippe J. Guerin[1], Stephen B. Duffull[3], Joel Tarning[1], Ric N. Price[1], Francois Nosten[1], Elizabeth A. Ashley[1], Arjen M. Dondorp[1], Rick M. Fairhurst[4], Duong Socheat[5], Steffen Borrmann[6], Anders Björkman[7], Andreas Mårtensson[7], Mayfong Mayxay[8], Paul Newton[1], Delia Bethell[9], Youry Se[9], Harald Noedl[10], Mahamadou Diakite[11], Abdoulaye A. Djimde[12], Nicholas J. White[1], Julie A. Simpson[2], Kasia Stepniewska[1]

[1]University of Oxford, Oxford, United Kingdom, [2]The University of Melbourne, Melbourne, Australia, [3]University of Otago, Otago, New Zealand, [4]National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [5]Entomology and Malaria Control, Pnom Penh, Cambodia, [6]Wellcome Trust Research Programme, Kilifi, Kenya, [7]Karolinska Institutet, Stockholm, Sweden, [8]Wellcome Trust-Mahosot Hospital-Oxford Tropical Research Collaboration, Vientiane, Lao People's Democratic Republic, [9]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [10]Medical University of Vienna, Vienna, Austria, [11]University of Bamako, Bamako, Mali, [12]University of Bamako, Bamako, Mali

The key pharmacodynamic measure of the artemisinin derivatives is the rate of parasite clearance in the days following treatment. Frequent assessments of the parasite density are needed to define this rate. The aim of this work was to apply two different methodologies to derive sampling schedules from which parasite clearance could be defined using the WWARN Parasite Clearance Estimator (PCE). The first approach used robust T-optimal design methodology to allow for discrimination across models that best describe an individual patient's parasite-time profile. The design was based on the constraint of no more than six samples per patient within 48 hours of initial treatment. The design was evaluated with a simulation-estimation procedure. The second approach selected 2744 real parasite-time patient profiles with 6 hourly counts in the first 48 hours. Bootstrapping was used to estimate the sampling distribution of half-lives (HL) from sampling schedules that excluded data from at least one of the 6-hourly sampling time-points (including the scheme 0, 6, 12, 24, then every 12 hours (A1)). A simulation study was performed to investigate 16 schemes, including scheme A1 and the T-optimal scheme. The T-optimal sampling times (sampling windows) were: 0 (0 to 2), 5.8 (4.0 to 6.0), 9.9 (8.0 to 12.0), 24.8 (24.0 to 24.9), 36.3 (34.0 to 38.0) and 48 (45.0, 48.0) hours post initial treatment. The simulation-estimation procedure showed that the design supported selection of the appropriate model for parasite clearance rate constant estimation. For the data-based approach, scheme A1 consistently performed the best, for a range of HLs. Of the reduced schemes, one with measurements at times[window] of 0[0-2], 6[4-8], 12[10-14], 24[22-26], 36[34-36], 48[46-50], or 6, 7, 24, 25, then every 24 hours (+ the subsequent hour) is recommended. Stopping sampling at 48 hours had little effect on the estimation of HL. The two methodologies produced consistent findings. The derived designs require further validation, but should be considered for future studies that intend to use the PCE.

823

IMPACT OF ARTEMISININ BASED COMBINATION THERAPY (ACTS) REPEATED TREATMENT ON THE PREVALENCE OF *PLASMODIUM FALCIPARUM* DRUG RESISTANCE MOLECULAR MARKERS, *PFCRT* AND *PFMDR1*

Aliou Traore, Demba Dembélé, Bakary Sidibé, Sékou Touré, Sekou Koumare, Amadou Togo, Kassim Sanogo, Ogobara Doumbo, Abdoulaye Djimde

Malaria Research and Training Center, Bamako, Mali

Based Combination Therapy (ACTs) are currently used as the malaria first-line treatment in most endemic countries. The aim of this study was to assess the impact of repeated treatment with AS + AQ and AR-L on *Pfcrt* and *Pfmdr1*, in a 3 years randomized clinical trial in Bougoula (Mali). We use WHO 28-day standard in-vivo protocol. Overal 521 blood spoted filter papers were analyzed; mutations frequencies on *Pfcrt* and *Pfmdr1* genes were compared before and after intervention. In the AS + AQ arm we observed a base line frequency of 41.6% against 77.1% for *Pfmdr1-86Y* during the first episode and > 93% in the second, third and fourth episodes of malaria. For the *Pfcrt76T gene* we observe a baseline frequency of 58.9% against 88% during the first episodes and > 93% in the next episodes. For the AR-L arm's, we obtained a baseline frequency of 41.6% against 6.2%, 18.2%, 7.1% and 0% on *Pfmdr186Y gene* for episodes 1, 2, 3 and 5 respectively. Concerning *Pfcrt76T* gene the base line frequency was 58.9% against 59.1%, 75% and 88.8% for episodes 1, 2 and 3 respectively. This study demonstrate that there is a significant increase in *Pfmdr-86Y*, and *Pfcrt-76T* mutants after treatment with AS + AQ and a significant decrease of *Pfmdr1* mutations after treatment with AR-L. Despite the presence of artemisinin,the CTAs select the molecular markers of resistance to the partner molecule.

824

POOLED DEEP SEQUENCING OF *PLASMODIUM FALCIPARUM PARASITEMIAS*: AN EFFICIENT AND SCALABLE TOOL TO QUANTIFY PREVAILING MALARIA DRUG-RESISTANCE GENOTYPES

Steve M. Taylor[1], Christian M. Parobek[2], Nash Aragam[3], Billy E. Ngsala[4], Andreas Martensson[5], Steven R. Meshnick[6], Jonathan J. Juliano[3]

[1]Duke University Medical Center, Durham, NC, United States, [2]University of North Carolina Department of Genetics, Chapel Hill, NC, United States, [3]University of North Carolina Division of Infectious Diseases, Chapel Hill, NC, United States, [4]Muhimbili University of Health and Allied Sciences, Dar es Salaam, United Republic of Tanzania, [5]Karolinska Institute, Stockholm, Sweden, [6]University of North Carolina Gillings School of Global Public Health, Chapel Hill, NC, United States

Molecular surveillance for drug-resistant malaria parasites requires efficient, timely, and scalable methods in order to provide actionable data. Genotyping parasite populations using second-generation sequencing may provide these data efficiently. We designed and validated a protocol to quantify the frequencies of mutant alleles associated with sulfadoxine-pyrmethamine resistance in *Plasmodium falciparum* genes dhfr and dhps in mixed *parasitemia*s using 454 sequencing. We applied this new protocol to field isolates collected from a cohort of 50 Tanzanian children with uncomplicated *falciparum* malaria, and compared it on accuracy and cost to standard genotyping methods that employ Sanger sequencing with or without statistical inference of allele frequencies. In validation experiments with a mixture of parasite strains 3d7 and V1/S, the 454 sequencing protocol accurately quantified dhfr and dhps allele frequencies. Using Sanger sequencing with statistical inference, the frequencies of mutant alleles in dhfr were 78.9% (dhfr51I), 80.5% (dhfr59R), 86.2% (dhfr108N), and 0 (dhfr164L); mutation frequencies in dhps were 58.5% (dhps437G), 51.1% (dhps540E), and 1.1% (dhps581G). 454 sequencing of pooled gDNA generated 91,157 and 92,638 reads of dhfr and dhps, respectively, which estimated mutant allele frequencies of 70.9% (dhfr51I),

84.6% (dhfr59R), 90.2% (dhfr108N), 0 (dhfr164L), 55.1% (dhps437G), 57.9% (dhps540E), and 6.2% (dhps581G); these estimates were highly correlated (>98%) with frequencies estimated by traditional methods. 454 sequencing obviated most molecular steps in traditional sequencing methods, and because of this would be cost-saving to generate allele frequencies for parasite population sizes larger than 50. This genotyping method based upon second-generation sequencing can efficiently and reproducibly estimate parasite allele frequencies within populations of *P. falciparum*. This method would be rapid and cost-effective for molecular epidemiologic studies of parasite genotypes associated with transmission, vaccine efficacy, and drug resistance.

825

GENOMIC EPIDEMIOLOGY OF ARTEMISININ RESISTANCE ON THE CHINA-MYANMAR BORDER

Fang Huang[1], Meera Venkatesan[2], Christopher G. Jacob[2], Shannon Takala-Harrison[2], Matthew Adams[2], Hui Liu[3], Henglin Yang[3], Shuisen Zhou[1], Linhua Tang[1], Christopher V. Plowe[2]

[1]National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention; World Health Organization Collaborating Centre for Malaria, Schistosomiasis and Filariasia; Laboratory of Parasite and Vector Biology, Ministry of Health, Shanghai, China, [2]Howard Hughes Medical Institute/University of Maryland School of Medicine, Baltimore, MD, United States, [3]Yunnan Institute of Parasitic Diseases, Puer, China

Emerging artemisinin resistance in Southeast Asia poses a significant risk to malaria control and eradication goals, including China's plan to eliminate malaria within its borders by 2020. Yunnan, China's only province with endemic *Plasmodium falciparum* malaria transmission, borders Myanmar, Vietnam and Laos, and is the key focus of the national malaria elimination program. Parasites from this region have shown decreased *in vitro* susceptibility to artemisinin and delayed parasite clearance after artemisinin treatment. Understanding the genetic basis of artemisinin resistance and identifying specific genetic *loci* associated with this phenotype is crucial for effective surveillance and containment of resistance. Parasites collected from 200 clinical trial participants from three field sites near the Myanmar border were genotyped using a *P. falciparum*-specific single nucleotide polymorphism (SNP) microarray. SNP profiles were examined for signatures of recent positive selection and prevalence of validated and candidate molecular markers of drug resistance. Population structure in each parasite population was evaluated by Principal Component Analysis and the program Structure, which uses a Bayesian framework for estimating sub-structure. Results of these genomic analyses will be discussed in the context of recent genome-wide association studies of genetic *loci* associated with artemisinin resistance. This study verifies the utility of DNA microarrays for large-scale parasite molecular epidemiology and will aid in the validation of candidate artemisinin resistance markers.

826

MONITORING THE EFFICACY AND SAFETY OF THREE ARTEMISININ COMBINATIONS THERAPIES (ACT) IN SENEGAL: RESULTS FROM TWO YEARS SURVEILLANCE

Khadime Sylla

Cheikh Anta Diop Dakar University, Dakar, Senegal

Malaria is a major public health problem in developing countries. The malaria control strategy includes the case management of clinical case. The obstacle of the case management of clinical malaria is the emergence and the spread of malaria drugs resistance. To cope with this situation WHO recommended the artemisinin combination therapies (ACT). After the scaling up of ACT in Senegal and the and the recent appearance of a decreased susceptibility of Pf to artemisinin based combination therapy in Asia, it becomes necessary to monitor the use of ACT in Africa west. The study was carried out in the transmission period of 2011 and 2012 in two health districts in Senegal. Study end points included (i) PCR corrected adequate clinical and parasitological response (ACPR) at day 28, (ii) ACPR at days 35 and 42, (iii) parasites and fever clearance time, (iv) incidence of adverse events and patients biological profile at day 7. The WHO 2003 protocol for antimalarial drug efficacy evaluation was used to assess each outcome. Three ACT were evaluated: dihydro-artemisinine-piperaquine DHAPQ (Duocotexcin*), artemether-lumefantrine AL (Coartem*) and artesunate-amodiaquine ASAQ. Overall, 534 patients were randomized to receive either DHAPQ (n=176), AL (n=178) and ASAQ (n=180). PCR corrected ACPR at day 28 was at 99.41% in ASAQ group while that was at 100% in DHAPQ and AL group (p=0.37). Therapeutic efficacy was at 100% in DHAPQ and AL group versus 99.37% in ASAQ group at day 35 (p=0.37). At day 42 ACPR at 100% was obtained in the DHAPQ and AL group versus 99.27% in ASAQ group (p=0.36).The three treatments were well tolerated with similar clinical and biological profile. In conclusion, Dihydro-Artémisinine-Pipéraquine (DHAPQ), Artemether-lumefantrine (AL) and Artesunate-Amodiaquine (ASAQ) are effectious and well tolerated for malaria treatment in Senegal mainly in West Africa in spite of the recent appearance of a decreased susceptibility of Pf to artemisinin based combination therapy in Asia.

827

EFFICACY OF THREE REGIMENS FOR UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN CAMBODIA

Dysoley Lek[1], Socheat Duong[1], Didier Menard[2], Agus Rachmat[3], William O. Rogers[3]

[1]National Center for Parasitology, Entomology and Malaria Control (CNM), Phnom Penh, Cambodia, [2]Institut Pasteur du Cambodge, Phnom Penh, Cambodia, [3]U.S. Naval Medical Research Unit - 2, Phnom Penh, Cambodia

Resistance to anti-malarial drugs, including artemisinin combination therapies is a growing problem. We assessed the efficacy of three therapies for uncomplicated *Plasmodium falciparum* malaria in Trapeang Prasat, Oddarmeanchey Province, Cambodia bordering Thailand. Sixty-three subjects with uncomplicated *P. falciparum* malaria received directly observed therapy with 12 mg/kg artesunate and 25 mg/kg mefloquine (A/M, over three days), up to a maximum dose of 600 mg artesunate/1250 mg mefloquine; 77 subjects received dihydroartemisinin-piperaquine (DP); 71 subjects received Malarone. Subjects were followed for 42 days or until recurrent *parasitemia* was observed. We used PCR genotyping of msp1, msp2, and glurp to distinguish treatment failure from new infections andtreatment failure rates at days 28 and 42 were analyzed with both per protocol and Kaplan-Meier. Real Time PCR was used to measure the copy number of the pfmdr gene and standard 48 hour isotopic hypoxanthine incorporation assays was used to measure IC_{50} for anti-malarial drugs. Fifty-two%, 62.3%, and 62.3% infected subjects were still parasitemic on day 3 respectively. The crude treatment failure rates at 28 days were 8%, 1.3%, and 4.3% respectively and at 42 days were 19%, 3%, and 6% respectively. Treatment failure was associated with increased pfmdr1 copy number for A/M and DP groups only; no difference noted for the malarone group. One subject in the A/M arm acquired new *P. falciparum* infection; the rest of recurrences were thought to be recrudescence. All four recurrent cases in the Malarone arm were found to be of cytochrome b wild type. In conclusion, the results support other studies that artesunate-mefloquine combination therapy continues to worsen in Northern Cambodia, even in the "tier 2 region," outside of containment zone 1 where A/M resistance was first described. It is unclear whether the treatment failures are due solely to mefloquine resistance or to artesunate resistance as well.

828

SEQUENCE AND COPY NUMBER POLYMORPHISMS IN THE MULTIDRUG RESISTANCE TRANSPORTER 1 *(PVMDR1)* GENE OF *PLASMODIUM VIVAX* PARASITES FROM VANUATU AND THE SOLOMON ISLANDS

Karryn J. Gresty[1], Edward Tambisari[2], Lasse S. Vestergaard[3], George K. Taleo[2], Lyndes Win[4], Albino Bobogare[4], Jeffrey Hii[5], Norman C. Waters[6], **Qin Cheng**[1]

[1]Australian Army Malaria Institute, Enoggera, Australia, [2]Vector Borne Disease Control Program, Ministry of Health, Port Vila, Vanuatu, [3]World Health Organization, Port Vila, Vanuatu, [4]Malaria and Vector Borne Diseases Control Program, Ministry of Health, Solomon Islands, [5]World Health Organization, Honiara, Solomon Islands, [6]Walter Reed Army Institute of Research, Division of Experimental Therapeutics, Silver Spring, MD, United States

In 2008, artemisinin-based combination therapy (ACT) was introduced as first-line treatment for confirmed cases of uncomplicated *Plasmodium falciparum* and *P. vivax* malaria in Vanuatu and the Solomon Islands, replacing chloroquine (CQ) and sulfadoxine-pyrimethamine (SP). The establishment of baseline drug resistance profiles allows for monitoring of the emergence of resistance to ACTs. Sequence polymorphisms in *pvmdr1* have been implicated in resistance to CQ and amodiaquine, while copy number polymorphisms in *pvmdr1* have been associated with resistance to mefloquine and lumefantrine. Using samples collected during therapeutic efficacy studies conducted in Epi Island, Vanuatu and Malaita Province, Solomon Islands, we investigated copy number and sequence polymorphisms in the *pvmdr1* gene of *P. vivax*. Results indicated that the majority of *P. vivax* parasites exhibited amino acid substitutions Y976**F** and F1076**L,** but retained only one copy of *pvmdr1*. This indicates *P. vivax* parasites in these islands are resistant to CQ and amodiaquine and a change of malaria treatment policy was timely.

829

ASSESSING THE COST-BENEFIT EFFECT OF A *PLASMODIUM FALCIPARUM* DRUG RESISTANCE MUTATION ON PARASITE GROWTH *IN VITRO*

Gabrielle Fröberg[1], Pedro Eduardo Ferreira[2], Doc Andreas Mårtensson[3], Abdullah Ali[4], Prof Anders Björkman[1], José Pedro Gil[5]

[1]Malaria Research Group, Karolinska Institutet, Stockholm, Sweden, [2]MTC, Karolinska Institutet, Stockholm, Sweden, [3]IHCAR/Malaria Research Group, Karolinska Institutet, Stockholm, Sweden, [4]Zanzibar Malaria Control Programme (ZMCP), Zanzibar, United Republic of Tanzania, [5]Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

Plasmodium falciparum mutations associated with antimalarial resistance may be beneficial for parasites under drug pressure, although they may also cause a fitness cost. We herein present an *in vitro* model showing how this combined effect on parasite growth varies with the drug concentration and suggest a calculated drug specific cost-benefit index, indicating the possible advantage for mutated parasites. We specifically studied *pfmdr1* D1246Y in relation to amodiaquine resistance. Susceptibilities to amodiaquine, desethylamodiaquine and chloroquine, as well as relative fitness were determined in two modified isogenic *P. falciparum* clones only differing in the *pfmdr1* 1246 position. Data were used to create a new comparative graph of the relative growth in relation to the drug concentration and to calculate the ratio between the benefit of resistance and the fitness cost. Results were related to an *in vivo* allele selection analysis after amodiaquine or artesunate-amodiaquine treatment. *Pfmdr1* 1246Y was associated with decreased susceptibility to amodiaquine and desethylamodiaquine, but at a growth fitness cost of 11%. Mutated parasites grew less in low drug concentrations due to a predominating fitness cost, but beyond a breakpoint concentration they grew more due to a predominating benefit of increased resistance. The cost-benefit indexes indicated that *pfmdr1* 1246Y was most advantageous for amodiaquine exposed parasites. In vivo a first drug selection of mutant parasites followed by a fitness selection of wild-type parasites supported the *in vitro* data. In conclusion, this cost-benefit model may predict the risk for selection of drug resistance mutations in different malaria transmission settings.

830

THE IMPACT OF MALARIA CONTROL INTERVENTIONS ON ANTIMALARIAL DRUG RESISTANCE: MEASURING THE ROLE OF CHANGES IN MULTIPLICITIES OF INFECTION

Aimee R. Taylor[1], Carol H. Sibley[2], Jennifer A. Flegg[1], Philippe J. Guerin[1], Chris Holmes[3], Method D. Segeja[4], Stephen M. Magesa[5], Insaf F. Khalil[6], Martha M. Lemnge[4], Ib C. Bygbjerg[6], Michael Alifrangis[6]

[1]WWARN - University of Oxford, Oxford, United Kingdom, [2]WWARN - University of Washington, Seattle, WA, United States, [3]University of Oxford, Oxford, United Kingdom, [4]National Institute for Medical Research, Tanga Research Centre, Tanga, United Republic of Tanzania, [5]RTI International, Nairobi, Kenya, [6]Centre for Medical Parasitology at Department of International Health, Immunology and Microbiology, Copenhagen, Denmark

To ensure effective malaria control strategies, it is important to investigate the impact of changing transmission on markers of antimalarial drug resistance, and thus genotypic multiplicity of infection. Genetic markers are often used to monitor antimalarial drug resistance, while multiclonal infections are largely ignored. However, interventions that reduce transmission intensity may also reduce the mean multiplicity of infection (MOI). The interpretation of changes in the prevalence of genetic markers in the face of changing MOIs is problematic, since prevalence is a function of the MOI. A Bayesian model, which uses a sampling algorithm, was developed to estimate genotype frequencies, which are comparable across different MOIs, and applied on a concrete study in an area with changing transmission: in 2003, an increase in prevalence of Pfdhfr wild type alleles after the introduction of insecticide treated nets (ITNs) in a village in Tanzania was reported, concurrent with a drop in the mean MOI. This paper addresses a major question: Did the decrease in the mean MOI alone result in changes in prevalence? Or did the differences reflect an increase in antimalarial susceptibility? Analyses based on statistically estimated genotype frequencies were compared with replicate analyses based on prevalence. Only small differences in the two approaches were detected, and they did not change the conclusion that the prevalence of susceptible alleles of Pfdhfr did increase after the introduction of ITNs. The authors' original proposal that 'lowering the level of transmission may be a method to indirectly increase sulfadoxine/pyrimethamine susceptibility by lowering drug use and, thus, drug pressure,' was substantiated, and the possibility that the increase in prevalence was a mere side effect of the drop in MOI ruled out.The new model has important implications for the surveillance of resistance in areas of decreasing malaria prevalence: by analysing frequency, estimates of resistance can be compared, and the confounding effect associated with changes in the MOI eliminated.

831

EFFICACY OF A THREE-DAY ARTESUNATE-MEFLOQUINE COMBINATION FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN MAE HONG SON, KANCHANABURI, UBONRATCHATHANI AND SURIN PROVINCES THAILAND IN 2011-2012

Apinya Niramitsantipong, Kanungnit Congpuong, Rungniran Sugaram, Wichai Satimai

Bureau of Vector - Borne Disease, Muang, Nonthaburi, Thailand

Current first-line treatment for *Plasmpdium falciparum* in Thailand is a 3-day artesunate-mefloquine combination. Study conducted in In 2009, the efficacy of the artesunate-mefloquine combination declined to 87-92.7% in 4 sentinel sites. In 2010 the efficacy in 4 sentinel sites revealed

efficacy of the 3-day artesunate-mefloquine combination between 90.9-98.0%). In view of the threat from *falciparum* parasites developing resistance to ACTs recently, monitoring the efficacy of the first line treatment to generate valuable information for updating the national treatment policy is critical. This study was undertaken to assess the efficacy and safety of artesunate-mefloquine for the treatment of uncomplicated *P. falciparum* malaria in Mae Hong Son, Kanchanaburi, Ubonratchathani and Surin provinces in Thailand Antimalarial drug efficacy trials will be conducted in 4 sentinel sites in Thailand for uncomplicated *falciparum* infection. The participants will be febrile patients aged 6 months and above with confirmed uncomplicated *P. falciparum* infection. Falciparum malaria patients will be treated with artesunate (4 mg/kg over 3 days) co-administered with mefloquine (15 mg/kg on day 1 and 10 mg/kg on day 2). Clinical and parasitological parameters will be monitored over a 42-day follow-up period to evaluate drug efficacy for *falciparum* malaria. The study will be conducted from March to December, 2012. The results of this study will be used to assist the Ministry of Public Health of Thailand in assessing the current national treatment guidelines for uncomplicated *P. falciparum* malaria. Completed follow-up uncomplicated *P. falciparum* patients include in the per protocol analysis were 8, 43, 8, and 2 in Mae Hong Son, Kanchanaburi, Ubonrajchathani and Surin province respectively. According to the WHO criteria LCF (PCR corrected) that found in following province were 0, 8, 0, 0 respectively. The cure rate (ACPR) in the following province were 100%, 72.42%, 87.5% and 100% and day 3 positive were 0%, 55.81%, 12.5% and 0% respectively. In conclusion, the efficacy of artesunate-mefloquine has declined in Kanchanaburi province. Continuing monitoring drug efficacy in this area should proceed and changing of anti-malarial drug regimen are consider.

832

PERCEPTION OF MALARIA RISK IN A SETTING OF REDUCED MALARIA TRANSMISSION: A QUALITATIVE STUDY IN ZANZIBAR

Julie A. Bauch

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

Malaria transmission has declined dramatically in Zanzibar in recent years. Continuing use of preventive measures such as long-lasting insecticidal-treated nets (LLINs), and use of malaria rapid diagnostic tests (RDTs) are essential to prevent malaria resurgence. This study employed qualitative methods to explore community perceptions of malaria risk and adherence to prevention measures in two districts in Zanzibar. Key informant interviews with 24 primary health care providers and 24 focus group discussions with local residents in Zanzibar districts Wete and Central were conducted during April and May 2012 focusing on perception of malaria risk, current preventive practices used, reasons for using preventive practices and effective strategies for malaria control. Health care providers and residents appear to be aware of the decreasing incidence of malaria. Both groups continue the use of malaria preventive practices in this low and seasonal transmission setting. The most important preventive measures identified were LLINs, indoor residual spraying (IRS), and education. Barriers to malaria prevention include: lack of staff at clinics, insufficient number of LLINs distributed, and inadequate malaria education. Reasons for continued use of preventive practices include: fear of malaria returning to high levels, presence of mosquitoes during rainy seasons, and concern about local cases from other villages or imported cases from mainland Tanzania. Mosques, clinics, schools and community meetings were listed as most important sources of education. However, residents express the desire for more education. Health care providers and residents generally reported consistent use of malaria preventive measures. However, maintaining and continuing to reduce malaria transmission will require ongoing education for both health care providers and residents to reinforce the importance of using preventive measures. Successful efforts to reduce malaria in Zanzibar will be jeopardized if residents believe that they are no longer at risk for malaria. In future studies, a year-round evaluation of the perception of malaria risk and use of preventive measures will inform the timing of education and prevention strategies for sustained malaria control.

833

FEBRILE ILLNESS MANAGEMENT IN CHILDREN UNDER FIVE YEARS OF AGE: A QUALITATIVE PILOT STUDY ON PRIMARY HEALTH CARE WORKERS PRACTICES IN ZANZIBAR

Mwinyi I. Msellem

Zanzibar Malaria Control Programme, Zanzibar, United Republic of Tanzania

In Zanzibar, malaria prevalence dropped substantially in the last decade and presently, most febrile patients seen in primary health care facilities (PHCF) test negative for malaria. The availability of RDTs allows rural health workers to reliably rule out malaria in fever patients. However, additional diagnostic tools to identify alternative fever causes are scarce, often leaving RDT negative patients without a clear diagnosis and management plan. This pilot study aimed to explore health workers' practices with febrile children and identify factors influencing their diagnostic and management decisions in non-malarial fever patients. Semi-structured key informant interviews were conducted with 12 health workers in six PHCFs in North A district, Zanzibar, April-June 2011. Interviews were coded using Atlas.ti to identify emerging themes that play a role in the diagnosis and management of febrile children. The following themes were identified: 1) Health workers use caregivers history of illness and RDT results for initial diagnostic and management decisions, but suggest caregivers need more education to prevent late presentation and poor health outcomes; 2) There is uncertainty regarding viral versus bacterial illness and health workers feel additional point-of-care diagnostic tests would help with differential diagnoses; 3) Stock-outs of medications and limited caregivers' resources are barriers to delivering good care; 4) Training, short courses and participation in research as well as; 5) Weather also influence the diagnostic decision-making. In conclusion, this pilot study found that health workers in Zanzibar use caregiver history of fever and results of malaria RDTs to guide management of febrile children. However, since most febrile children test negative for malaria, health workers believe additional training and point-of-care tests would improve their ability to diagnose and manage non-malarial fevers. Educating caregivers on signs and symptoms of febrile illness, as well as the introduction of additional tests to differentiate between viral and bacterial illness, would be important steps to get children to PHCFs earlier and decrease unnecessary antibiotic prescribing without compromising patient safety. More research is needed to expand our understanding of what would improve fever management in other resource-limited settings with decreasing malaria.

834

PLASMODIUM VIVAX HIGH-THROUGHPUT-LOOP MEDIATED ISOTHERMAL AMPLIFICATION (HTLAMP): IMPROVING THE DIAGNOSTIC TOOLKIT FOR MALARIA ELIMINATION

Sumudu Britton

Queensland Institute of Medical Research, Brisbane, Australia

Plasmodium vivax is the most geographically widespread of the *Plasmodium* species and poses formidable challenges to achieve elimination. Loop-mediated isothermal amplification (LAMP) holds potential as a sensitive molecular diagnostic technique for the identification of sub-patent malaria infection. However, its deployment is currently limited by the absence of a sufficiently sensitive species-specific assay for *P. vivax* infection, and by the absence of a platform suitable for high-throughput analysis in the field. Here we describe a new high-throughput, colourimetric, field applicable *P. vivax* LAMP(htLAMP) assay targeting the *P. vivax* mitochondrial COX1 gene. The assay was adapted to a 96-well plate colourimetric high-throughput format, and evaluated for sensitivity and specificity. The assay was performed in a 65^0 C waterbath with visually detectable colour-change results confirmed in an ELISA plate

reader. A *P. vivax* sample of known *parasitemia* (based on real-time PCR) was used to prepare a two-fold dilution series to establish the limit of detection of the assay. HtLAMP was then compared to nested PCR-based diagnosis on bloodspots extracted using saponin and chelex-based rapid DNA extraction protocols, to establish sensitivity and specificity. The limit of detection of the *P. vivax* htLAMP assay was 1.3 parasites/µL of packed red blood cells on DNA extracted from whole blood. The assay did not cross react with DNA from other human species of plasmodia except *P. knowlesi*. The htLAMP assay was then compared with nested PCR on a clinical sample set (n=42), showing a sensitivity of 100%, specificity of 48%, PPV 46% and NPV 100%. The assay turnaround time was 1 hour (following DNA extraction). In conclusion, this new *P. vivax* htLAMP assay shows promise as a sensitive, species-specific diagnostic tool for rapid detection of sub-patent *P. vivax* infections, and is amenable to epidemiologic and elimination activity in *vivax*-endemic settings.

835

PERSISTENT SUB-MICROSCOPIC *PLASMODIUM FALCIPARUM* WITH MUTANT CRT AND MDR1 GENOTYPES, A PROBLEM FOR MALARIA ELIMINATION IN THE PROVINCES OF SARANGANI AND TAWI-TAWI ON THE ISLANDS OF MINDANAO, PHILIPPINES

Mary Grace Dacuma[1], Federico Yadao[2], Walter Notario[3], Antonio Yasaña[4], Ernesto Bona[5], Judeline Dimalibot[6], Sukarno Asri[7], Virgilio Mori[8], Fatima Allian[8], Rachel Hallett[1], Colin Sutherland[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Malaria Control Program, Provincial Health Office, Sarangani Province, Philippines, [3]Pilipinas Shell Foundation, Sarangani Province, Philippines, [4]Provincial Health Office, Sarangani Province, Philippines, [5]Department of Health Region XII, Sarangani Province, Philippines, [6]University of the Philippines Los Baños, Laguna, Philippines, [7]Provincial Health Office, Bongao, Tawi-Tawi, Philippines, [8]Sahaya Sin Kawman, Bongao, Tawi-Tawi, Philippines

The Philippines has set a national goal to eliminate malaria by 2020. In line with this, the country adopted artemether-lumefantrine (AL) as first-line treatment option for *Plasmodium falciparum* malaria in 2009. However, *P. falciparum* resistance to previously used antimalarials in the country such as chloroquine and amodiaquine might affect the usefulness of AL in the Philippines. In this study, the prevalence of *P. falciparum* was investigated in malaria endemic provinces of Sarangani and Tawi-Tawi in Mindanao, Philippines. *P. falciparum* was found in 1.8% (16/907) and 1.9% (21/1088) of cross-sectional survey participants by nested PCR using DNA extracted from dried blood spots on filter paper. These isolates were genotyped for previously reported polymorphisms in the chloroquine-resistance transporter (crt) gene and multi-drug resistance (mdr1) gene using real-time PCR, nested PCR and sequencing. At present, crt codons 72-76 have been analyzed in 14 and 21. *P. falciparum* isolates from Sarangani and Tawi-Tawi, respectively. Three of the 14 isolates from Sarangani (21.4%) harboured wild-type CVMNK, five isolates (35.7%) were CVIET, five (35.7%) were SVMNT, and one isolate was a mixed infection of CVMNK and CVIET. Four of the 21 isolates from Tawi-Tawi (19%) were CVMNK, one was CVIET, and 15 (71.4%) were SVMNT, with one mixed infection of CVMNK and CVIET. This was the first report for presence of *P. falciparum* with CVIET haplotype in Mindanao, Philippines. Both CVIET and SVMNT haplotypes have been previously associated with chloroquine and amodiaquine resistance. When the 16 *P. falciparum* isolates from Sarangani were genotyped for polymorphisms at mdr1 codons 86 and 184, 50% (8/16) have 86Y while 100% have 184F. Of the 11 out of 21 *P. falciparum* isolates from Tawi-Tawi genotyped to date for polymorphisms at mdr1 codons 86 and 184, 27.3% (3/11) have 86Y while 100% have 184F. These findings raised two issues relevant to malaria elimination in Sarangani and Tawi-Tawi: (1) the persistence of sub-microscopic *P. falciparum* circulating in human population despite existing control measures; and (2) the occurrence of *P. falciparum* with both CVIET or SVMNT haplotypes and mdr1 mutations are present in Mindanao.

836

USE OF PASSIVE AND ACTIVE SURVEILLANCE TO ASSESS THE ROLE OF HOUSING AS A POTENTIAL RISK FACTOR FOR LOCAL TRANSMISSION OF *PLASMODIUM FALCIPARUM* INFECTION IN SWAZILAND

Nyasatu E. Ntshalintshali[1], Zulisile Zulu[2], Joseph M. Novotny[1], Simon P. Kunene[3], Patrick Msibi[4], Hugh Sturrock[5], Iveth Gonzalez[6], David Bell[6], Grant Dorsey[5], Roly Gosling[5], Bryan Greenhouse[5], Michelle S. Hsiang[5]

[1]Clinton Health Access Initiative, Mbabane, Swaziland, [2]National Malaria Control Programme, Manzini, Swaziland, [3]National Malaria Control Programme, Mbabane, Swaziland, [4]National Reference Laboratory, Ministry of Health, Mbabane, Swaziland, [5]University of California San Francisco, San Francisco, CA, United States, [6]FIND Diagnostics, Geneva, Switzerland

Understanding the role of housing as a potential risk factor for local transmission in Swaziland may highlight new areas for intervention as the country aims for malaria elimination. As part of a prospective study on active surveillance (investigation of index cases with screening their households and neighbors) we evaluated the relationship between housing and symptomatic or asymptomatic infection acquired locally. Symptomatic infection among index cases was diagnosed by *Plasmodium falciparum* Rapid Diagnostic Tests or microscopy and asymptomatic infection by Loop-mediated isothermal amplification (LAMP). The comparison group was LAMP-negative subjects screened in active case detection. Information about risk factors was collected through interviews and housing data collected by examination. To analyze relationships between infection and risk factors, logistic regression was performed. From Aug 2012 to Jan 2013, 197 malaria cases were reported in passive surveillance. Of 136 cases that were investigated, 30 were classified as locally acquired based on travel history. Of 779 household members and neighbors of index cases screened in active case detection, LAMP was completed in 477 and 8 infections were identified (1.68%). Compared to subjects living in a house with unscreened windows or no windows, mud, cane, grass or shrub internal or external walls, and a grass or palm roof, subjects living in a house with screened windows, cement block or brick external walls, cement block or brick or plaster internal walls, and a roof with metal sheets or tile had 5.2 higher odds of infection, 95% CI 1.6-17.1. Infection was also associated with reported travel within Swaziland, OR 2.42, 95% CI 1.0-5.8. There were no associations with age, sex, region, occupation, travel outside Swaziland, use of an insecticide treated bed net, or sleeping under a structure sprayed with insecticide in the past year. Adjusting for travel within Swaziland, higher quality housing had a 5.2 higher odds of infection, 95% CI 1.6-17.2. Unlike in other studies, poor quality housing was not found to be associated with infection. It is possible that in this low transmission setting, poor housing is not associated with infection. The association between higher quality housing and infection may be confounded by some other risk factor such as travel within Swaziland. Also, as our study is nascent and has not yet included wet season data, sample sizes for infected subjects were small.

837

MALARIA CASES AMONG MIGRANT WORKERS AT THE HYDRO-POWER PROJECT SITE IN BHUTAN

Dechen Pemo, Tashi Tobgay, Rinzin Namgay

Vector - Borne Disease Control Program, Gelephu, Bhutan

There was a drastic decline in malaria cases in Bhutan with 12,591 cases and 22 deaths in 1999 to just 82 cases and one death in 2012. This decline has prompted program to shift from control strategies to elimination phase. Therefore, as the country moves into the elimination phase, monitoring the importation of cases is vital and imperative to prevent introduction of parasite into the areas where malaria vectors are prevalent and climatic conditions are suitable for transmission. Hence, this screening was carried out as a routing surveillance system to find malaria

parasites in migrant workers at two large hydropower projects in Bhutan. A total of 5797 migrant workers at two project site, Punatsangchu (4594 workers) and Mangdechu (1203 workers) were screened for plasmodium infections. Blood samples were examined using microscopy by the trained malaria microscopists over the period of 45 days and the positive cases were re-confirmed by rapid diagnostic test kits (RDT). There were eight positives cases with four cases *Plasmodium falciparum* and 4 cases *P. vivax*. Majority of workers were from West Bengal (27.77%). Most of the positive cases were from Jharkhand (4 cases). Among the positive cases only one patient had fever. Entomological surveillance found the presence of *Anopheles pseudowillmori*, *Aedes aegypti*, *Ae. albopictus* and sandfly in and around the project area. This finding indicates a high risk of malaria transmission and also other vector borne diseases as a result of parasite introduction from migrant workers. Since there are number of migrant workers from malaria ,dengue and kala-azar endemic states of India, there is a dire need to strengthen monitoring and surveillance of these workers to minimize disease transmission in the locality

838

ASSESSMENT OF INDOOR DENSITIES AND INFECTIOUSNESS OF MALARIA VECTORS IN THREE VILLAGES IN ULANGA DISTRICT, SOUTHERN TANZANIA

Emanuel W. Kaindoa

Ifakara Health Institute, Morogoro, United Republic of Tanzania

High quality mosquito surveillance data is necessary to; assess spatial and temporal disease trends, evaluate new and existing interventions, identify transmission hotspots, identify dominant vectors and pathogens or detect new ones, to readily detect disease outbreaks and predict future disease trends. We established a longitudinal adult mosquito surveillance system in rural Tanzania, to provide essential data necessary for examining: 1) indoor densities of disease transmitting mosquitoes in the area, 2) prevalence of *Plasmodium* sporozoite among malaria vector populations in the area, and 3) the baseline malaria transmission intensity in the study area. From a population of 2433 households in 3 villages (Kivukoni, Minepa and Mavimba) in southern Tanzania, 1600 households were randomly selected and spatially assigned, based on latitudes, to 16 clusters each consisting of 100 households. Monthly mosquito collections were performed using CDC-Light traps inside 6 households randomly selected from each cluster. The mosquitoes were sorted by taxa and abdominal status, after which a sub-sample of the malaria vectors were examined by (PCR) to distinguish between sibling species. The vectors were also examined by (ELISA) to detect *Plasmodium* sporozoite in their salivary glands. Densities of the two main malaria vectors, *Anopheles gambiae* s.l and *An. funestus* were significantly associated with latitude (the cluster of sampling) ($df = 15$, $P<0.001$) and month of the year when the sampling was done ($df = 11$, $P<0.001$).The distribution of *Culex* mosquitoes was such that areas with the lowest densities of *Anopheles* were also the areas with the highest densities of *Culex*. There was also a clear temporal variation in densities of mosquitoes, the peak densities being observed around April and May. For *An. funestus* however, we also observed that the mean indoor catches tended to be higher during the dry season (starting May to August than the rest of the year.) The distribution of *An. gambiae* s.l was spatially clustered, mostly in a set of adjoining clusters centered on the middle of the study area while densities of *An. funestus* was higher in the southern part of the study area than in the rest of the area thus suggesting suitability of spatially targeted intervention.

839

COMMUNITY PERSPECTIVES AND PRACTICES RELATED TO OUTDOOR MALARIA TRANSMISSION IN RURAL TANZANIA

Irene R. Moshi, Edith Madumla and Fredros Okumu

Ifakara Health Institute, Ifakara, United Republic of Tanzania

Indoor malaria prevention methods such as Long-Lasting Insecticide Treated Nets have significantly reduced the disease burden in Africa, yet transmission persists in many communities, partly driven by mosquitoes that bite people outdoors. It is essential to consider perspectives of local communities towards this outdoor transmission, so as to inform the development of new tools for malaria control. We assessed views and behaviors of rural and peri-urban communities in southern Tanzania, regarding outdoor mosquito bites and malaria prevention. A cross-sectional survey was conducted in two rural and two peri-urban villages in southern Tanzania, using semi-structured interviews and structured observations. A total of 40 households were studied. The interviews assessed whether malaria vectors also bite outdoors and transmission can also occur outdoors, while the observations were used to identify common outdoor activities that expose people to mosquito bites and current means of protection against the outdoor bites. A prototype outdoor mosquito control device was then used to assess community responses towards such potential outdoor interventions in future malaria control. More than 90% of the respondents knew about malaria and had regularly experienced outdoor mosquito bites and complained of malaria persistence, but most of them still believed that transmission occurs mostly indoors such that some use repellents or long cloths to prevent themselves from biting. Common outdoor activities included shopping and socializing (30% of people observed), storytelling (21%), cooking (18%), eating (16%), fetching water (15%), all of which took place between 6pm and 11pm, and also starting 5am and 6:30 am, matching times when outdoor biting mosquitoes are also known to be most active. The respondents were willing to use and contribute towards financing of the outdoor devices for malaria control. In conclusion, the results show that people appreciate outdoor biting and the likelihood of outdoor transmission but use of intervention other than bed nets indoors, was rare. Providing well developed outdoor mosquito control devices that are acceptable will contribute substantially in reducing malaria transmission.

840

USING COMMUNITY KNOWLEDGE AND EXPERIENCES TO PREDICT DENSITIES AND DISTRIBUTION OF DISEASE-TRANSMITTING MOSQUITOES IN RURAL TANZANIA

Stephen P. Mwangungulu

Ifakara Health Institute, Morogoro, United Republic of Tanzania

The current lack of reliable techniques that can be used for large scale programmatic monitoring of distribution and densities of disease transmitting mosquitoes is a major challenge to public health authorities, especially in low and middle income endemic countries. We describe here a new community-based participatory mapping approach that relies simply on the knowledge and experiences of residents to rapidly identify areas where disease transmitting mosquitoes are most abundant. The method is proposed for use in spatial targeting of mosquito control interventions. Such simplified methodologies for mapping outdoor vector densities will be particularly necessary for optimal placement of outdoor mosquito control devices, such as odour-baited mosquito traps or lure and kill stations. Step 1: Develop participatory representative maps of the study areas showing essential landmark features, which can be used by community members to classify mosquito densities. Step 2: Selected community members are asked to identify locations where they think mosquitoes are most abundant, by ranking the grids on a scale of 1-5. Step 3: The data generated is interpolated and classified to show places where people think there are high, medium of low mosquito densities. Step 4: Entomological sampling is conducted to verify outdoor mosquito densities in locations identified by community members as having high, medium and low mosquito densities. Step 5: Validation of the community based perceptions and data obtained from entomological sampling. Maps were derived from community knowledge and opinions on the mosquito density distributions. The maps were generated by interpolating the grid ranks as provided by the community members. The data is reclassified to show places identified having High, Medium and Low Mosquito densities. Interpolation was done using Inverse Distance Weighting method and Outdoor entomological sampling was conducted in areas that were identified by community members as having, High, Medium or Low

densities of Mosquitoes distributions. This study provides evidence that we can rely on community knowledge and experience to identify suitable areas where mosquitoes are most abundant and where to locate outdoor complementary interventions .Such method will be cheaper, quicker and easier and potentially will guide large scale implementation of outdoor control devices for lure and kill disease-transmitting mosquitoes.

841

TREATMENT OF ASYMPTOMATIC CARRIERS OF *PLASMODIUM FALCIPARUM* WITH ARTEMETHER-LUMEFANTRINE: IMPACT ON THE PREVALENCE OF ANEMIA

Alfred B. Tiono[1], Alphonse Ouedraogo[1], Christine Remy[2], Kamal Hamed[3]

[1]Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso, [2]Novartis Pharma AG, Basel, Switzerland, [3]Novartis Pharmaceuticals Corporation, East Hanover, NJ, United States

The effect of systematic treatment of asymptomatic carriers of *Plasmodium falciparum* with artemether-lumefantrine (AL) on hemoglobin (Hb) levels and anemic status, compared with no treatment of asymptomatic carriers, was investigated in a 12-month, single-center, controlled, parallel, cluster-randomized study in 18 villages in Burkina Faso. Intervention and control village inhabitants participated in three community screening campaigns (CSC1-3) that took place approximately one month apart before the rainy season, and a fourth campaign (CSC4) after the rainy season had ended to mark the end of the study at12 months. The change in Hb level in all asymptomatic carriers aged >6 months from Day 1 to Day 28 of CSC1 was +0.53 g/dl (from 11.81 to 12.33 g/dl) in the intervention arm vs -0.21 g/dl (from 12.06 to 11.86 g/dl) in the control arm ($p<0.001$). During the same period, the proportion of asymptomatic carriers aged >6 months to <5 years with anemia (mild, moderate or severe) in the intervention arm decreased by 31.1% (from 75.7% to 44.6%), compared with a decrease of 4.7% (from 76.3% to 71.6%) in the control arm. After 12 months, the proportion of asymptomatic carriers with anemia was reduced in both arms. Systematic screening and treatment of asymptomatic carriers of *P. falciparum* with AL at the community level can reduce the prevalence of anemia in children in the short term (28 days), although the difference with the control arm was not maintained at 12 months.

842

QUANTIFYING MALARIA RE-INTRODUCTION RISK IN NAMIBIA IN A POST-ELIMINATION SETTING

Nick Ruktanonchai[1], Trevor Caughlin[1], Zhuojie Huang[2], Udayan Kumar[1], Amit Verma[3], Christopher Lourenco[4], Andrew J. Tatem[5], David L. Smith[6]

[1]University of Florida, Gainesville, FL, United States, [2]Center for Infectious Disease Dynamics, Pennsylvania State University, University Park, PA, United States, [3]Center for Disease Dynamics, Economics and Policy, Washington, DC, United States, [4]Southern Africa Malaria Elimination Support Team, Clinton Health Access Initiative, Windhoek, Namibia, [5]University of Southampton, Southampton, United Kingdom, [6]Johns Hopkins University, Baltimore, MD, United States

For the many countries pursuing malaria elimination, reintroduction risk from neighboring countries is a major concern. One of these countries is Namibia, which is nearing malaria elimination, but shares borders with countries with endemic malaria. A quantitative framework for predicting spatial risk of epidemic spread upon reintroduction could enable policymakers to more efficiently implement country-wide surveillance and control methods. We develop such a quantitative framework by combining spatial data on malaria transmission with a unique dataset on human movement between Namibian settlements. We hypothesize: 1) some settlements will be particularly susceptible to reintroduction, 2) some settlements will be epidemic sinks, or locations where epidemic spread is especially likely, and 3) these settlements can be predicted using a combination of transmission intensity and human movement metrics. We used an agent-based model (ABM) to simulate malaria transmission and assess likely paths of epidemic spread across 402 settlements in Namibia. Movement patterns were parameterized using a dataset consisting of mobile phone records for 1,191,000 people in Namibia, and potential transmission intensity was estimated using prevalence estimates for each settlement. Overall epidemic likelihood was low, due to low transmission rates country-wide. However, epidemic likelihood varied greatly dependent on the settlement where reintroduction occurred, ranging from 0% to 40% of reintroductions yielding an epidemic. In some cases, settlements close in proximity and similar in transmission intensity differed greatly in reintroduction risk, due to movement patterns of people in the settlements. Although urban areas have low malaria transmission, we found that certain commerce centers, such as Windhoek and Oshakati, were likely to receive malaria during an epidemic. These results help inform surveillance efforts in post-elimination strategic planning, and provide an understanding of the settlement characteristics involved in yielding and promoting spread of malaria epidemics.

843

ACTIVE SURVEILLANCE WITH REALAMP TO FIND ASYMPTOMATIC MALARIA INFECTIONS IN KANCHANABURI PROVINCE, THAILAND

Jaymin C. Patel[1], Kanungnit Congpuong[2], Wichai Satimai[2], Steven R. Meshnick[1]

[1]University of North Carolina at Chapel Hill, Chapel Hill, NC, United States, [2]Bureau of Vector-Borne Diseases, Ministry of Public Health, Nonthaburi, Nonthaburi, Thailand

Early and accurate diagnosis of asymptomatic malaria infections is essential for malaria control programs, especially for countries focusing on elimination. Currently, active malaria surveillance in Thailand relies on microscopy or rapid diagnostic tests (RDTs); however, both methods have suboptimal sensitivity and specificity to detect low-density *parasitemia*s. As Thailand aims to eliminate malaria, improved methods for detecting submicroscopic malaria infections are needed. RealAmp integrates loop mediated isothermal amplification (LAMP) with fluorescence detection and can be done on a portable, battery operated device. A field study was undertaken in June 2012 to determine the utility of the RealAmp assay in detecting asymptomatic cases via active surveillance in Kanchanaburi province near the Thai-Myanmar border. Microscopy slides and dried blood spots were collected from 127 healthy individuals from 2 villages and a local school. Microscopy slides were examined by a microscopist at the local malaria clinic and an expert microscopist at the Bureau of Vector Borne Disease laboratory in Bangkok. Genomic DNA was extracted using 20% Chelex. All samples were tested in duplicate by real-time pooled PCR and RealAmp genus assay. A total of 7 positive samples (5 *P. vivax*, 1 *P. malariae*, 1 *P. falciparum* and *P. vivax* mixed infection) were identified by the reference pooled PCR method. The RealAmp assay correctly identified all 7 infections (sensitivity = 100% 95%CI: 59-100% and specificity = 97% 95% CI: 92-99%) while local and expert microscopy identified 3 and 4 infections respectively. The median age among those who tested positive was 22 years and 4 out of 7 were females. A majority (71%) of the positive cases were migrants living in Thailand and all but one reported agriculture as their primary occupation. Active surveillance using RealAmp detected more asymptomatic cases than either local or expert microscopy. These results have significant implications for local elimination efforts as migrant populations can introduce and facilitate transmission. RealAmp may provide an alternative to real-time pooled PCR as means to rapidly and efficiently detect asymptomatic malaria infections, which serve as important reservoirs.

844

DOES TYPHOID FEVER AWAKEN THE HYPNOZOITES OF *VIVAX* MALARIA?

G. Dennis Shanks[1], Nicholas White[2]

[1]Army Malaria Institute, Enoggera, Australia, [2]Mahidol Oxford Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Persons with a single infection may develop another especially in the tropics during the pre-antibiotic era. When examining the old tropical medicine textbooks it was found that several authors (Laveran, Reed, Craig, James) noted the co-incidence of typhoid fever and *vivax* malaria relapse. Since fever has been postulated to trigger *vivax* relapses, we sought examples from the medical literature that might clarify if typhoid fever activates latent liver parasites (hypnozoites). Reed reported a series of 12 cases in US Army soldiers who had returned to the USA from Cuba in 1900 who had malaria relapses confirmed by microscopy following typhoid fever. Malaria relapses were noted to occur after becoming afebrile from typhoid. Simultaneous paratyphoid C and malaria epidemics were reported by Giglioli in an aluminum mining population in Guyana in 1926-27. Twenty-nine percent of paratyphoid patients also had *vivax* malaria on presentation and 32 / 82 cases (39%) died; one third of all deaths in hospital during the 1926 / 27 epidemic had both infections. During a 1928 typhoid epidemic in Fajardo, Puerto Rico there were 90 typhoid cases in a town of 15000. Of the 63 typhoid cases reviewed, 11 / 63 (17%) also had malaria parasites in their blood. During the Second World War in North Africa, 10 of 34 patients (29% of British soldiers, Italian Prisoners of War and Sudanese civilians) with typhoid fever also had *vivax* malaria. Typhoid fever can be associated with *vivax* relapses but proof of a causal relationship has not yet been found.

845

ASSESSMENT OF CLIENT SATISFACTION WITH INTEGRATED COMMUNITY MANAGEMENT PROGRAM: WAKISO, UGANDA

Tumuhamye Nathan

Makerere University, Kampala, Uganda

Malaria, pneumonia and diarrhea are the leading causes of death in children under five in Uganda. In a bid to improve access to prompt effective treatment of these diseases, Malaria Consortium in partnership with United Nations International Children's Emergency Fund (UNICEF) introduced Integrated Community Case Management (ICCM) in eight central districts of Uganda. There is currently limited information on client satisfaction with this program. The main objective of this study was to assess client satisfaction with the ICCM program in Wakiso district, one of the implementing sites. A cross sectional survey using a modified SERQUAL tool was used to investigate client satisfaction with the ICCM program. The study population consisted of randomly selected care givers of children under the age of five years who had used ICCM services. Four hundred fifty four caregivers of children below five participated in the study. Data was collected using semi-structured interviews translated in Luganda after pre-testing the tool. Data was analyzed in STATA version10. Logistic regression was used to determine the association between client satisfaction and several factors. Among 454 respondents, 80% of the care givers of children under five were satisfied with ICCM program. The overall gap (-0.332) between expectations and perceptions was significant, (t=-4.89, p-value 0.0081) meaning that despite the high level of client satisfaction, there still exist a quality gap in services provided under ICCM. Furthermore, there were no significant differences in the expectation and perception scores among the different dimensions except for reliability which had a score of -0.49 (p-value 0.0005). The multivariable logistic regression model showed that primary education (OR 2.8, 95% CI 1.116-6.795) and being a Muslim (OR 2.9, 95% CI 1.403372-6.341) was significantly associated with client satisfaction. Overall, 80% of the clients were satisfied with ICCM services and there was no statistical significant difference between perceptions and expectations for all the dimensions except for reliability dimension. However, there is a quality gap in the services provided under ICCM services in Wakiso district. The DHO and implementing partners should study these gaps critically and develop relevant strategies to improve on the quality of ICCM services in regard to these variables.

846

RECALCULATING THE NET USE GAP: A MULTI-COUNTRY COMPARISON OF ITN USE VERSUS ITN ACCESS

Hannah M. Koenker

JHUCCP, Baltimore, MD, United States

Use of insecticide treated nets (ITN) is widely recognized as one of the main interventions to prevent malaria and high use rates are a central goal of malaria programs. The difference between household ownership of at least one ITN and population use of ITN has in the past been seen as evidence for failure to achieve appropriate net use. We review data from the last seven years to recalculate the net use gap using the comparison indicator of 'access to nets within the household' as now recommended by RBM and WHO. Data from 36 DHS and MIS surveys (2005-2012) in sub Saharan Africa were used. For each dataset three indicators were calculated: access to and use of ITN by the population and household ownership of at least one ITN. Access was calculated by multiplying the number of ITNs in the household by 2 to obtain potential users in the household. This value was then divided by the de-facto members setting the value to 1 if potential users exceeded members. Use gap was expressed as the ratio between use and access. The range of values for household ownership of ITN was 3.5-90.9%, for access 1.5-74.5% and ITN use 0.3-68.4%. There was a close, linear relationship between access and ownership ($p<0.0001$, R-squared 0.93) showing the access result was on average 32% lower than ITN ownership. Overall the median proportion of users compared to those with access was 85.2% (Interquartile Range 71.5% to 99.6%) with 8 surveys (22%) showing proportions below 70% (range 11.2% to 69.4%) and another 8 surveys with a result above 100% (range 102% to 119%) indicating mean users per net exceeded 2.0 in these cases. Even at access levels <50% a median 81.2% used an ITN given they had access and this rate increased to 96.4% for access rates >50%. Linear regression of use against access showed an estimated use of 90.1% (95% CI 84.8-95.3) given access. However, the variation of use was high at lower access values and significantly decreased with increasing access (test for heteroskedasticity $p=0.008$) indicating more consistent use at higher access rates. These results clearly show that previous interpretations of low use rates as a failure of behavioral change communication to use nets was not justified and that low use rates were driven by lack of ownership instead. They also demonstrate the usefulness of the newly recommended distinction between use and actual access to ITN.

847

THE NATIONAL MALARIA PREVALENCE IN HAITI IS <1%

Roland Oscar[1], **Kimberly E. Mace**[2], Samuel E. Jean[3], Alexandre Existe[4], Michelle Chang[2], Jean Frantz Lemoine[1], Eniko Akom[3]

[1]Programme National de Contrôle de la Malaria, Port au Prince, Haiti, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Population Services International - Haiti, Port au Prince, Haiti, [4]Laboratoire National de la Santé Publique, Port au Prince, Haiti

Malaria is endemic in Haiti, although a recent prevalence estimate is not available. In December 2011, during the rainy season, a national, population-based Tracking Results Continuously survey was conducted, funded by Global Fund, to estimate malaria prevalence. A cross-sectional, two-stage cluster design was utilized where enumeration areas (EAs) were selected with a probability proportional to size and 20 households (HHs) within EAs were randomly selected. Questionnaires were conducted and all HH members were tested for malaria using three methods: rapid diagnostic tests (RDTs), microscopy by Haiti's reference laboratory, and polymerase chain reaction (PCR). RDTs were performed for 3944 persons,

16 (0.41%) were positive for *Plasmodium falciparum* (*Pf*) antigen and were treated. Of 3055 microscopy results, 12 (0.39%) were found with *Pf* parasites. Results from 2989 PCR tests showed 13 (0.43%) were positive. The number of persons positive for malaria by any single test was 27 and these were distributed among Haiti's ten Departments as follows: 11 (41%) Artibonite, 8 (30%) Ouest (3 in metropolitan Port au Prince); 3 (11%) Nord; 2 (7%) Nord Ouest, 2 (7%) Sud; and 1 (4%) Grand Anse. No other species of *Plasmodium* was found. The median age of the 27 test-positive persons was 6 years; range 1-51 years. Experiencing fever in the previous two weeks was reported for 18 (67%) persons; of whom 10 reported previous treatment with chloroquine. Nine of 27 (33%) had asymptomatic malaria, defined as the detection of *Pf* by any method and no reported fever in the previous two weeks. While the 3 testing methods produced similar estimates of malaria prevalence, there were discordant results between methods on the same samples. In order to monitor program progress over time, future surveys should include oversampling in areas suspected of higher transmission and using serology, a more sensitive test, to characterize malaria epidemiology in this low transmission setting. Malaria prevalence in Haiti is low and heterogeneous; these findings are encouraging for future elimination efforts.

848

EVALUATING THE IMPACT OF MALARIA INTERVENTIONS IN NIGERIA

Ndukwe K. Ukoha, Azra Ghani, Maria-Gloria Basáñez

Imperial College, London, London, United Kingdom

Malaria control has intensified globally with targets set by the World Health Assembly (WHA); the Roll Back Malaria (RBM) Partnership and the Millennium Development Goals (MDGs). These targets aim for the short-term goal of reducing the burden of malaria until it is no longer a public-health problem, and the long-term goal of reducing the global incidence to zero by progressively eliminating the disease in endemic countries.International funding for intervention scale up has risen steeply in the past decade. Goals are becoming more ambitious as countries record reductions in malaria incidence. In spite of this progress, Africa still bears the enormity of the global burden of the disease, with Nigeria accounting for a quarter of the disease burden in the continent. A review of evaluation studies on malaria interventions show that the link between intervention scale up and decreasing trends in malaria incidence and deaths in Nigeria remains unclear. This lack of clarity between intervention scale up and decreasing trends in malaria incidence underscores the need for this project, which seeks to evaluate the impact of scaled-up intervention coverage over the past decade in Nigeria. Data on prevalence, morbidity and mortality were extracted systematically from baseline evaluation studies of malaria transmission in Nigeria from the year 2000 onwards. These data were geo-located, classified bystate, and statistically analysed. Subsequently, intervention coverage data on mosquitonets, Artemisinin-based combination therapy rates and Intermittent Preventive Treatment were collated and evaluated. Descriptive analysis was undertaken on the intervention coverage data to characterise the scale up and to uncover spatial variation in interventions uptake. The results of these analyses informed the design of a survey to understand the impact of intervention scale up by region, and gaps between expected and observed impact. This survey focused on areas in which impact has been achieved and those in which further impact could be made to understand barriers to intervention effectiveness. Whilst the final results of this work is being aggregated, the main objective is to provide evidence-based recommendations to the NMCP regarding ways in which malaria control can be further improved in Nigeria, thereby offering further steps towards achieving the WHA, the RBM Partnership and the MDG targets for malaria in Nigeria.

849

ANTI MSP1 AND AMA-1 ANTIBODY DYNAMICS AND THEIR INFLUENCE ON THE OCCURRENCE OF SYMPTOMATIC EPISODES OF MALARIA IN A COHORT OF CHILDREN AGED BELOW FIVE YEARS

Ben Andagalu, Edwin Kamau, Bernhards Ogutu

KEMRI Walter Reed Project, Kisumu, Kenya

MSP-1 and AMA -1 are key antigens that are produced during the asexual stages of the plasmodium life cycle. The relationship between the levels of antibodies to these antigens and their effects on control of *parasitemia* levels and protection from clinical disease is unclear. We studied the dynamics of these antibody levels and their influence on *parasitemia* levels and on the occurrence of symptomatic episodes of malaria in a subset of children from a cohort study that was conducted in Western Kenya between 2003 and 2004. A total of 270 asymptomatic children aged between 12 months and 47 months were randomized to receive either a course of Artemether Lumefantrine (Coartem) or placebo and then followed up for 1 year. Active surveillance consisted of weekly visits by field workers and monthly visits at the study clinic, during which laboratory assessments were carried out. Passive surveillance consisted of unscheduled visits. Immunological data was available for a subset of 60 children. The anti-AMA-1 and anti-MSP-1 antibody levels at baseline were higher in the older children. The youngest age category had an overall median anti-AMA-1 level of 2.8ug/ml compared to 31.8ug/ml and 100.5ug/ml in the older age categories. The overall anti-MSP-1 antibody levels showed a different trend, with the youngest age category having a median of 1.0ug/ml compared to 0.43ug/ml and 0.65ug/ml in the 24-35 month and 36-47 month age categories. The differences in median levels across age categories was statistically significant.There was a strong correlation between anti-AMA-1 antibody levels and *parasitemia* levels, and separately between anti-MSP and *parasitemia* levels. Lower antibody levels seen in subjects experiencing *parasitemia* levels ≥5000/ul as compared to those experiencing lower levels of *parasitemia*, and those with negative smears having the lowest antibody levels. Episodes of asymptomatic *parasitemia* were associated with higher anti-AMA-1 and anti-MSP-1 antibody levels in comparison with symptomatic malaria episodes and absence of parasites. This association was statistically significant. High anti-AMA-1 and anti MSP-1 antibody levels at baseline were not associated with a significant reduction in the number of clinical malaria. Similarly, the overall antibody levels were not associated with a significant reduction in the number of clinical malaria episodes.

850

DECLINING BURDEN OF MALARIA OVER TWO DECADES IN A RURAL COMMUNITY OF MUHEZA DISTRICT, NORTHEASTERN TANZANIA

Deus Ishengoma[1], Bruno Mmbando[1], Method Segeja[1], Michael Alifrangis[2], Martha Lemnge[1], Ib Bygbjerg[2]

[1]National Institute for Medical Research, Tanga, United Republic of Tanzania, [2]Centre for Medical Parasitology, University of Copenhagen, Copenhagen, Denmark

The recently reported declining burden of malaria in some Africa countries has been attributed to scaling-up of different interventions although in some areas, these changes started before implementation of major interventions. This study assessed the long term trends of malaria burden for 20 years (1992 - 2012) in Magoda and for 15 years in Mpapayu village of Muheza district, North-eastern Tanzania, in relation to different interventions as well as changing national malaria control policies. Repeated cross-sectional surveys recruited individuals aged 0 - 19 years from the 2 villages whereby blood smears were collected for detection of malaria parasites by microscopy. Prevalence and density of *Plasmodium falciparum* infections and other indices of malaria burden (prevalence of splenomegaly and gametocytaemia) were compared across the years and between the study villages. Major interventions and changes in

malaria control policies were also marked. In Magoda, the prevalence of *P. falciparum* infections initially decreased between 1992 and 1996 (from 83.5 to 62.0%), stabilized between 1996 and 1997, and further declined to 34.4% in 2004. A temporary increase between 2004 and 2008 was followed by a progressive decline to 7% in 2012, i.e. > 10-fold decrease since 1992. In Mpapayu (from 1998), the highest prevalence was 81.5% in 1999 and it decreased to 25% in 2004. After a slight increase in 2008, a steady decline followed, reaching <5% from 2011. Bed-net usage was high in both villages from 1999 to 2004 (≥88%) but it decreased between 2008 and 2012 (range, 28 to 68%). After adjusting for the effects of bed-nets, age, fever and year of study, the risk of *P. falciparum* infections decreased significantly, by ≥97% in both villages between 1999 and 2012 (p40% to <1%) and gametocytaemia (23% to <1%) also decreased markedly in both villages. In conclusion, a remarkable decline in the burden of malaria occurred between 1992 and 2012. The initial decline (1992 - 2004) was possibly due to the deployed interventions while the steady decline observed from 2008 (with bed-net coverage) suggests that other factors contributed to these changes. These results provide evidence that could be used to monitor progress towards elimination of malaria as a public health problem and reaching related MDGs.

851

COMPARISON OF AGE-SPECIFIC MALARIA MORTALITY RATES IN THE KEMRI/CDC HEALTH AND DEMOGRAPHIC SURVEILLANCE SYSTEM IN WESTERN KENYA, 2003-2010

Meghna Desai[1], Ann M. Buff[2], Sammy Khagayi[3], Annemieke van Eijk[4], Laurence Slutsker[5], John Vulule[6], Frank O. Odhiambo[3], Kayla F. Laserson[7], Mary J. Hamel[5]

[1]Kenya Medical Research Institute/Centers for Disease Control and Prevention Research and Public Health Collaboration, Kisumu, Kenya, [2]Centers for Disease Control and Prevention, Nairobi, Kenya, [3]Kenya Medical Research Institute/Centers for Disease Control and Prevention Research and Public Health Collaboration and KEMRI Center for Global Health Research, Kisumu, Kenya, [4]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [5]Centers for Disease Control and Prevention, Atlanta, GA, United States, [6]KEMRI Center for Global Health Research, Kisumu, Kenya, [7]Centers for Disease Control and Prevention, New Delhi, India

Malaria control interventions have been scaled-up in Kenya during the past decade. Most malaria interventions and burden assessments have focused on young children. Modeling of malaria burden in adults has produced highly variable estimates. To measure progress, accurate malaria burden estimates across age groups are necessary. We determined age-specific malaria mortality rates in western Kenya, an area of high malaria prevalence (38%, 2006), HIV prevalence (15% in adults, 2003-2004) and insecticide-treated net use (41%, 2006). We collected data from 140,000 persons in a health and demographic surveillance system from 2003-2010. Deaths were captured via community informant reporting and thrice yearly surveillance visits. Standardized verbal autopsies were conducted; probable cause of death was assigned by InterVA-4, a probabilistic method for verbal autopsy interpretation. Annual malaria mortality rates per 1,000 person-years (PY) were generated by age group. Trends from 2003-2010 were analyzed using Poisson regression. In children <5 years, the malaria-specific mortality rate (MMR) decreased from 13.2 to 3.7 per 1,000 PY, a mean reduction of 20% annually from 2003 to 2010. In children 5-14 years, MMR remained stable from 0.46 to 0.47 per 1,000 PY. In adults ≥15 years, MMR decreased from 1.5 to 0.42 per 1,000 PY for a mean reduction of 20% annually. From 2003 to 2010, the proportion of all malaria deaths by age group decreased in children <5 years from 69.8% to 61.4%, increased in children 5-14 years from 4.3% to 14.3% and decreased slightly in adults 25.9% to 24.3%. Malaria mortality rates in young children and adults have decreased dramatically from 2003 to 2010 in western Kenya, but older children have not benefited. Almost 40% of malaria deaths occur in older children and adults. Malaria deaths in adults might be overestimated due to HIV-associated mortality. Our data support current strategies to reach older children and adults and inclusion of all age groups in malaria control interventions, including universal coverage with insecticide-treated nets.

852

HOME INSPECTIONS TO VALIDATE CAREGIVER-REPORTED USE OF INSECTICIDE-TREATED BED NETS IN MACHINGA DISTRICT, MALAWI

Jacklyn Wong[1], Monica Shah[1], Dyson Mwandama[2], John E. Gimnig[1], Kim A. Lindblade[1], Don P. Mathanga[2]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Malaria Alert Centre, University of Malawi, Blantyre, Malawi

Studies evaluating use of insecticide-treated bed nets (ITNs) rely on self- or caregiver-reported use because direct observation at night is usually not possible. Such reports may be inflated, however, due to recall bias or social pressure to use ITNs, resulting in underestimates of true ITN effectiveness in reducing malaria risk. Following caregiver interviews conducted at centralized locations, we validated caregiver-reported ITN use through visual inspection of nets at the household. As part of a malaria cohort study in children (ages 6-59 months) in Machinga District, Malawi, caregivers were interviewed each month regarding their child's history of ITN use, including ITN use on the previous night. Between December 2012 and January 2013, a simple random sample of enrolled children (n=173) was selected and their caregivers were visited at their home within three days of the initial monthly interview by a surveyor who was blinded to their initial report of ITN use. The surveyor asked if the child used an ITN the previous night and, if the caregiver responded yes, requested to see the child's ITN hanging inside the house. The response from home inspections was scored as positive only if the child's ITN was visually observed. Due to problems with heavy rains and access to homes, median time from initial interview to home inspection was four days (range 0 to 14 days). Out of the 173 selected children, 30 had not slept in their own home the night before the initial monthly interview and were removed from analysis. For the remaining 143 children, 141 (98%) caregivers reported use of an ITN the night prior to the initial interview. Similarly, 141(98%) caregivers provided access to their child's ITN during the home inspection. However, 4 (2%) caregivers gave discordant responses in the home inspection compared to the initial interview. Considering the home inspection as the gold standard, the sensitivity of caregiver report was 98.6%. Due to the high level of ITN use, specificity could not be calculated. Results from caregiver reports and home inspections showed a high degree of agreement, indicating that ITN use patterns are high and consistent in Machinga District.

853

MALARIA IN PREGNANCY, LOW BIRTH WEIGHT AND PRETERM DELIVERY IN OUELESSEBOUGOU, MALI

Moctar Tounkara[1], Youssoufa Sidibe[1], Moussa Traore Traore[1], Amadou Barry[1], Almahamoudou Mahamar[1], Oumar Attaher[1], Souleymane Diarra[1], Yahia Dicko[1], Abdoulbaki Diallo[1], Gaoussou Santara[1], Djibrilla Issiaka[1], Michal Fried[2], Patrick E. Duffy[2], Alassane Dicko[1]

[1]Mali Research and Training Center, Faculty of Medicine, Pharmacy and Dentistry, University of Science, Techniques and Technologies (USTT), Bamako, Mali, [2]Laboratoryof Malaria Immunology and Vaccinology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

Pregnant women are at high risk of malaria infection that can lead to poor pregnancy outcomes including preterm delivery and low birth weight. To determine the frequency of malaria in pregnant women, and the risk factors for low birth weight and preterm delivery in Ouelessebougou, we enrolled pregnant women in a cohort study and followed them up to delivery. Blood smear was performed at enrollment, again between 30 and 32 weeks of gestation, on the day of delivery, and at the time of any

illness. Pearson's chi square test or Fisher's exact probability were used for comparison of proportions, and logistic regression was used for analysis of risk factors. 46.3 % (357/665) of pregnant women experienced malaria infection during pregnancy. The risk of pregnancy malaria (PM) was higher in primigravidae (OR= 2.41 [95% CI 1.62; 3.58]) and secundigravidae (OR= 1.74 [95% CI 1.15; 2.63]), compared to multigravidae. Use of ITN reduced the risk of PM by half (OR= 0.49 [95% CI 0.34; 0.70]). The proportion of low birth weight babies (LBW) was 9.6 % (61/636). Compared to multigravidae, the risk of LBW was higher in primigravidae (OR=3.84 [95% CI 1.98; 7.46]) and secundigravidae (OR= 2.39 [95% CI 1.17; 4.94]), adjusting for other factors such as use of intermittent preventive treatment with Sulfadoxine -Pyrimethamine (IPT -SP) and used of ITN. The frequency of preterm delivery was 10.4% (67/645). The risk of preterm delivery was higher for primigravidae (OR= 5.8 [95% CI 2.98; 7.46]), followed by secundigravidae (OR= 2.5 [95% CI 1.16; 5.28]). Use of IPT -SP was associated with reduced risk of preterm delivery (OR = 0.50 [95% CI 0.27; 0.84]). In conclusion, factors associated with malaria during pregnancy in Ouelessebougou were primiparity and use of ITN. Primiparity was also associated with the occurrence of low birth weight, while IPT-SP reduced the risk of preterm delivery.

854

MAPPING ARTEMISININ RESISTANCE: SMART SURVEILLANCE

Jennifer A. Flegg, The WWARN Smart Surveillance Study Group

University of Oxford, Oxford, United Kingdom

Artemisinin resistance has been detected in several *loci* in South East Asia. Current studies are ongoing to measure the extent of the spread. However, due to limited resources and the complexity of the methods used to characterize resistance, the number of sites remains limited. A methodology is proposed that will allow candidate sites to be chosen in an informed way and serve as a proof of concept for "Smart Surveillance" in general. Using malaria endemicity and human population density estimates in the Mekong region, along with maps of uncertainty in resistance based on resistance data, we aim to assess the effect of adding additional candidate sites to the dataset. The objective is to find the candidate sites that most reduce the uncertainty and maximize the useful information. Using parasite clearance half-life (HL) estimates from studies conducted in the Mekong region to characterize artemisinin resistance, the data are dichotomized in that we define the number of 'positive' responses as those with a HL above a cutoff. The cutoff is defined on a distribution of HLs in populations expected to be as sensitive to artemisinin as those observed in Africa. We develop a Bayesian geostatistical model of the proportion of individuals with a HL more than the cutoff. The model is fitted using a Markov chain Monte Carlo (MCMC) simulation and a predictive map is generated on a regular grid of the Mekong region, using the samples from the MCMC. For each prediction location, probabilities of the HL being greater than the cutoff are drawn and the distribution summarized as the median of this set. The associated uncertainty maps that accompany the median maps are created by calculating the coefficient of variation. The methods outlined here could play an important role in identifying where future efficacy studies should be done and inform decisions on surveillance of artemisinin resistance and elimination in the Mekong region.

855

CELL-MEDIATED IMMUNITY TO *PLASMODIUM FALCIPARUM* MEROZOITE SURFACE PROTEIN-1 IN KENYAN CHILDREN

Natalie M. Bowman[1], Jonathan J. Juliano[1], Chandy C. John[2], Carole A. Long[3], Steven R. Meshnick[1], John Vulule[4], Ann M. Moormann[5]

[1]University of North Carolina-Chapel Hill, Chapel Hill, NC, United States, [2]University of Minnesota, Minneapolis, MN, United States, [3]National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [4]Centre for Global Health Research, Kenya Medical Research Institute, Nairobi, Kenya, [5]University of Massachusetts Medical School, Worcester, MA, United States

Merozoite surface protein-1 (MSP1) is an abundant, immunogenic blood-stage protein and a malaria vaccine candidate. In order to compare the development and maintenance of immunity to MSP1, we analyzed immune responses to T cell epitope regions from MSP1 allelic variant (3D7 and FVO) in 164 Kenyan children living in Kisumu County, a lowland region with holoendemic malaria, and Nandi County, a highland region with hypoendemic malaria. Samples collected in February 2003 and November 2004 were analyzed for *parasitemia* by microscopy, cell-mediated immunity by interferon-γ ELISPOT, and MSP1 genotype (3D7 vs. FVO) by polymerase chain reaction of the M33 fragment in block 16 of the gene. In 2003, 79% of Kisumu children and 12% of Nandi children were parasitemic; in 2004, 87% of Kisumu children and only 1 (1%) in Nandi were parasitemic. Based on sequencing of block 16 of MSP1, the 3D7 genotype was more common at both sites, detected in 48% and 73% of parasitemic samples in Kisumu and Nandi respectively. In Kisumu, ELISPOT positivity to 3D7 increased from 2003 to 2004 from 33% to 40% and magnitude increased by 72%; in Nandi, prevalence declined from 45% to 17% and magnitude declined by almost 75%. Odds of positive ELISPOT increased with age. ELISPOT reactivity was protective against concurrent *parasitemia* at both sites in 2003 (summary OR 0.45, 95% CI 0.22, 0.93) and in Kisumu in 2004 (OR 0.28, 95% CI 0.04, 1.50). In multivariate analyses, positive ELISPOT in 2004 was associated with older age, concurrent *parasitemia*, and past positive ELISPOT, though with the exception of age, confidence intervals included 1. These results suggest that cell-mediated immunity to MSP1 is protective against concurrent *parasitemia* but is short-lived, with immunity waning over time in the absence of continuous exposure to malaria.

856

AN INVESTIGATION OF MALARIA INTERVENTION IN MOZAMBIQUE THROUGH MATHEMATICAL MODELING

Deidra J. Bowman, Helene Carabin

University of Oklahoma Health Sciences Center, College of Public Health, Oklahoma City, OK, United States

Malaria has been a significant cause of morbidity and mortality in many Sub-Saharan African countries, especially in children under five years. A mathematical model was developed that could demonstrate malaria prevalence in Mozambique, and an intervention strategy was applied to the model to determine the effectiveness of the intervention. A modified Anderson-May compartmental model of the malaria disease-burden in Mozambique during 2008 was generated using differential equations in Berkeley-Madonna software by applying data obtained from census reports and literature review. The model split the human compartment into two groups, children under five years of age and all other ages, to reflect differences in malaria mortality related to lack of immunity in younger children and partial immunity in those over five-years of age. Intervention goals for insecticide treated net (ITN) usage as proposed by the President's Malaria Initiative (PMI) were applied to the model to test for effectiveness in reducing prevalence in the under-five population. Malaria prevalence and the basic (R_0) and net effective (R_n) reproductive numbers were measured. The model predicted the malaria prevalence among children under-five years to be 8% of the population, or approximately 1,790,000

cases; the prevalence among the over-five population was 24%, or 4,455,000 cases. R_0 in the older population was 9. R_0 in children under-five years was 1,142. After introducing 85% ITN usage, the prevalence in the under-five population had been reduced to 5.2%, or 1,163,916. The R_0 was reduced to 26 due to a decrease in mosquito bite rate. The R_n increased to 7.8 due to a higher number of susceptible in the population. The current intervention goal of 85% ITN usage temporarily reduced malaria prevalence by 2.9% in the children under five. This would delay onset of illness to an age when mortality from illness would be decreased. Multiple intervention methods could further reduce prevalence.

857

USE OF A TRIANGULATION APPROACH TO ASSESS URBAN MALARIA IN GHANA

Edward Bonku[1], Bob Pond[2], Paul Psychas[3], Martin Adjuik[4], Pida Worlanyo[5], Katherine Beal[2], David O'Brien[2]

[1]JSI Research & Training Institute, Inc., Accra, Ghana, [2]JSI Research & Training Institute, Inc., Boston, MA, United States, [3]Centers for Disease Control and Prevention, Accra, Ghana, [4]INDEPTH NETWORK Secretariat, Accra, Ghana, [5]School of Public Health of the University of Ghana, Accra, Ghana

Public health decisions in Ghana have been informed by routine health service data suggesting that malaria is highly endemic throughout the country. A triangulation methodology was used to assess more rigorously the burden of malaria in the largest cities of the country. National stakeholders identified key questions which were addressed through analysis of existing data from household surveys (including the 2011 Multiple Indicator Cluster Survey - MICS), routine data from the Ghana Health Service (GHS), and previously published entomologic and epidemiologic research (including data accessed from the web site of the Malaria Atlas Project). Data were assigned to specific communities based upon geo-coordinates (where available) or the district in which they were collected. GHS data showed that 82% of all reported malaria cases and 95% of Accra cases were diagnosed without laboratory confirmation. Data from the MICS demonstrated that 80% of children in rural areas aged 5-59 months with a recent fever had a malaria infection (by rapid diagnostic test- RDT) but only 7% of febrile children from Accra were RDT positive. Published research as well as data from the MICS show that children in the largest cities of the three ecological zones were significantly less likely to be infected compared to children living in smaller communities of the same zone: 86% (95% CI: 66-94%) less likely in Accra vs. the rural coastal zone; 85% (95% CI: 66-93%) less likely in Kumasi vs. the rural forest zone; and 68% (95% CI: 37-84%) less likely in Tamale vs. the rural savannah zone. Laboratory confirmation of malaria is least common in Accra, where the prevalence of malaria is the lowest of any area of the country and where presumptive diagnosis is least reliable. This method, as yet little used in the malaria field, provides an evidence base for discussion among stakeholders and decision-making.

858

MOLECULAR EPIDEMIOLOGY OF PFCRT MUTANT HAPLOTYPES IN THE DEMOCRATIC REPUBLIC OF CONGO

Alejandro L. Antonia[1], Steve M. Taylor[1], Antoinette K. Tshefu[2], Mark Janko[3], Michael Emch[3], Steven R. Meshnick[1]

[1]University of North Carolina Gillings School of Global Public Health, Chapel Hill, NC, United States, [2]Ecole de Sante Publique, Faculte de Medicine, Kinshasa, Democratic Republic of the Congo, [3]University of North Carolina Department of Geography, Chapel Hill, NC, United States

Mutations in the *Plasmodium falciparum* chloroquine resistant transporter (pfcrt) mediate resistance to multiple antimalarials including chloroquine (CQ) and amodiaquine (AQ). Despite the widespread prevalence of these mutations, chloroquine and amodiaquine remain possible alternatives to or partners with artemisinin drugs for treatment of *falciparum* malaria. Molecular surveillance for haplotypes conferring resistance to these drugs is necessary to understand the feasibility of the re-deployment of these drugs in practice. In order to describe the epidemiology of these genetic markers in the Democratic Republic of Congo (DRC), we genotyped parasites from 180 individuals sampled in the 2007 Demographic and Health Survey (DHS) using PCR amplification across codons 72-76 of pfcrt and direct sequencing. 166 (92.2%) samples were successfully genotyped. The wildtype haplotype CVMNK was present in 79 (47.6%), the chloroquine-resistant CVIET haplotype in 55 (33.1%), a mixture of CVMNK and CVIET in 31 (18.7%), and CVMDK in one (0.6%). Overall, 86 *parasitemia*s (51.8%) harbored the pfcrt K76T substitution which is highly correlated with *in vitro* and *in vivo* drug failure. The SVMNT haplotype, which is associated with reduced susceptibility to amodiaquine, was not observed. Geographic analysis of the distribution of parasites bearing CVMNK and CVIET haplotypes indicated that parasite populations are sympatric without clear geographic clustering. According to DHS data, chloroquine accounted for 19.4% and amodiaquine 15.3% of antimalarials administered for childhood fever; on a provincial level, there were no associations between prevalences of mutant haplotypes and the use of either drug. In the DRC, we document molecular evidence of substantial CQ resistance but no mutations associated with AQ resistance, suggesting contrasting durability of these antimalarials. This cross-sectional study of the genetic landscape of pfcrt in the DRC highlights the importance of continued surveillance of genetic markers of drug resistance to effectively inform public health policies.

859

IMPACT OF INCREASED DISTRICT-LEVEL INSECTICIDE-TREATED NET (ITN) DISTRIBUTION ON ALL-CAUSE UNDER-FIVE MORTALITY IN MALAWI, 2004-2010

Lia S. Florey[1], Adam Bennett[2], Cameron Taylor[1], Christine Hershey[3], Achuyt Bhattarai[4], Carrie Nielsen[4], S. Rene Salgado[3], Doreen Ali[5], Misheck Luhanga[5], Thomas Eisele[2], Yazoume Ye[1]

[1]ICF International, Calverton, MD, United States, [2]Center for Applied Malaria Research and Evaluation, Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States, [3]United States Agency for International Development - President's Malaria Initiative, Washington, DC, United States, [4]Centers for Disease Control and Prevention - President's Malaria Initiative, Atlanta, GA, United States, [5]National Malaria Control Program, Ministry of Health, Lilongwe, Malawi

Insecticide treated mosquito nets (ITN) have been shown to be highly effective at reducing malaria morbidity and mortality in children. However, there are limited studies that assess the association between increasing ITN distribution and child mortality over time at the district level and under programmatic conditions. We used a Poisson model to assess the association between ITN program intensity and reductions in all-cause mortality in children less than five years of age in Malawi during 2004-2010 using district platform analysis. District-level ITN to population ratios were estimated using annual district ITN distribution data with a decay factor to account for loss of ITNs due to any cause (physical and/or chemical) in the previous years and mid-year population from census data. These ratios were then grouped into two categories representing low (<0.25) and high (0.25-1.2) ratios of ITNs available per person. Data from the 2006 Multiple Indicator Cluster Survey and the 2010 Demographic and Health Survey were used to construct annual district-level estimates for potential confounders; weighted averages were constructed for interim years. A multivariate generalized linear model with Poisson distribution controlling for household access to improved water sources, mother's educational level, prevalence of diarrhea, percentage of children under-five who were given vitamin A in the past six months, *Plasmodium falciparum* prevalence rate in children 2-10 years of age ($Pf PR_{2-10}$), and annual rainfall anomalies was constructed. In this model, higher district ITN program intensity was significantly associated with lower all-cause under-five mortality (incidence rate ratio = 0.85; 95% CI = 0.75-0.97). These findings suggest that increasing ITN distribution may have significantly contributed

to the decline in all-cause under-five mortality during 2004-2010 in Malawi and represent a novel use of district-level data from nationally-representative surveys.

860

FORECASTING MALARIA IN UGANDA WITH AGE AND ENVIRONMENTAL PREDICTORS

Kate Zinszer[1], Ruth Kigozi[2], Katia Charland[1], Grant Dorsey[3], Moses Kamya[4], David Buckeridge[1]

[1]McGill University, Montreal, QC, Canada, [2]Uganda Malaria Surveillance Project, Kampala, Uganda, [3]University of California San Francisco, San Francisco, CA, United States, [4]Makerere University, Kampala, Uganda

Accurate predictions of malaria could provide public health and clinical health services with an opportunity to develop proactive, targeted approaches for malaria control and prevention that make effective use of limited resources. The objective of the research was to develop and evaluate the accuracy of malaria forecasting models for six different sentinel sites in Uganda, using clinical and environmental predictors. Health clinic data collected by the sentinel site surveillance program, Uganda Malaria Surveillance Project, was used in conjunction with satellite-derived rainfall, temperature, and vegetation estimates. Weekly, site-specific time series were created and examined for all variables. The potential lag between each predictor and confirmed malaria was incorporated into the Autoregressive Integrated Moving Average models. We generated a set of short-term, intermediate, and long-term forecasts of malaria prevalence at weekly intervals and the average forecast error was calculated, using a reserved portion of the training series. The temporal interaction of the environmental predictors and malaria ranged from one week to four months. The significance of rainfall, temperature, and vegetation as predictors differed across the sites, although generally, temperature and vegetation were better predictors of malaria when compared to rainfall. The forecasting accuracy improved with the series stratified by age group and the environmental predictors were significant for malaria prevalence for ages 0-5 and 5-15 but less often significant for ages 15 and over. The forecasting accuracy deteriorated with increased forecasting intervals and the short-term forecast accuracy ranged from 5% to 29% across the sites (mean absolute percent error). Our work demonstrates the utility of using environmental covariates for the prediction of malaria, when used in conjunction with historical counts of malaria stratified by age. Future work includes examining the predictive influence of treatment and malaria screening practices which we anticipate will improve the forecast accuracy of our models.

861

MALARIA EPIDEMIOLOGY IN FORESTED AREA OF CENTRAL VIETNAM: THE HIDDEN PARASITE RESERVOIR

Thanh Pham Vinh[1], Hong Nguyen Van[1], Carine Van Malderen[2], Anna Rosanas-Urgell[3], Van Nguyen Van[4], Umberto D'Alessandro[5], Duong Tran Thanh[1], Niko Speybroeck[2], Annette Erhart[3]

[1]Vietnam National Institute of Malariology, Parasitology and Entomology, Hanoi, Vietnam, [2]Université Catholique de Louvain, Bruxelles, Belgium, [3]Institute of Tropical Medicine Prince Leopold (ITM), Antwerp, Belgium, [4]Provincial Malaria Station (Tam Ky city), Quang Nam, Vietnam, [5]Medical Research Council Unit (MRC), Fajara, Gambia

After successfully reducing the malaria burden to pre-elimination levels over the past two decades, Vietnam has recently engaged into a national malaria elimination program. However, in forested areas of Central Vietnam malaria elimination is likely to be jeopardized by the high occurrence of asymptomatic and sub-microscopic infections as shown by previous reports. We present the results of a malaria survey carried out in a remote forested area of Central Vietnam where malaria prevalence and risk factors were evaluated. After a full census (4 study villages=1,810 inhabitants), the study population was screened for malaria infections by standard microscopy and, if needed, treated according to national guidelines. An additional blood sample on filter paper was also taken in a random sample of the population for later polymerase chain reaction (PCR) and more accurate estimation of the actual burden of malaria infections. The risk factor analysis for malaria infections was done using survey multivariate logistic regression as well as the classification and regression tree method (CART). A total of 1,450 individuals were screened. Malaria prevalence by microscopy was 7.8% (ranging from 3.9 to 10.9% across villages): mostly *Plasmodium falciparum* (>81.4%) or P.*vivax* (17.7%) mono-infections; the large majority (69.9%) was asymptomatic. By PCR, the prevalence was estimated at 22.6%, (ranging from 16.4% to 42.5%) with a higher proportion of *P. vivax* mono-infections (43.2%). The proportion of sub-patent infections increased with increasing age and with decreasing prevalence across villages. The main risk factors were age (<30y), village, house structure, and no bed net. This study confirmed that in Central Vietnam a substantial part of the human malaria reservoir is hidden. Additional studies are urgently needed to assess the contribution of this hidden reservoir to the maintenance of malaria transmission. Such evidence will be crucial for guiding elimination strategies.

862

ASSESSING COST OPTIMIZED STRATEGIES FOR MAINTAINING AND EXTENDING THE GAINS AGAINST MALARIA

Charlotte Dolenz[1], Arnaud Le Menach[1], Elsa Hansen[2], Adam Bennett[3], David Smith[4], Justin Cohen[1], Bruno Moonen[1]

[1]Clinton Health Access Initiative, Boston, MA, United States, [2]Harvard School of Public Health, Boston, MA, United States, [3]Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States, [4]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Malaria burden has been substantially reduced in many endemic regions as a result of concerted efforts to scale-up insecticide-treated nets and effective treatment. Yet it remains unclear whether universal coverage of vector control will be required to maintain these gains, and whether permanent campaigns are feasible given the challenges of sustaining financing and preventing resistance. Alternatively, vector control may be withdrawn without risk of resurgence if sufficient fractions of malaria infections are cured, either through mass treatment or after being identified via case detection. Here, we describe a framework for defining the required surveillance infrastructure for a specific context that can identify and cure the fraction of infections required to maintain the gains or end transmission in the absence of continued vector control. Using the epidemiological contexts of Zambia and Haiti, we first estimate the intrinsic, spatially-varying risk of malaria by producing maps of R_0, the expected number of infections per infected human that would result in the absence of interventions. Second, we apply a mathematical transmission model to assess the fraction of infections that must be cured in order to maintain current prevalence or reduce it to zero. Third, we assess operational surveillance strategies that would be needed to achieve this threshold, including both passive and active measures. Fourth, we examine the relative costs of required surveillance strengthening compared to ongoing vector control. Initial results from Zambia suggest that prevalence can be maintained in areas where risk is very low ($R_0 < 3$) if active case detection supplements passive surveillance to identify and cure 30-60% of infections. In Haiti, strengthened passive detection combined with active measures, potentially including mass screening and/or treatment campaigns in transmission foci, will likely be sufficient to achieve elimination. Results suggest investment in surveillance-driven strategies may prove substantially cheaper and more sustainable than ongoing vector control.

863

ASSOCIATION BETWEEN INCREASING ITN USE AND REDUCTIONS MODERATE-TO-SEVERE ANEMIA IN CHILDREN 6 - 23 MONTHS OF AGE: A MULTI-COUNTRY DECOMPOSITION ANALYSIS

Lia S. Florey, Cameron Taylor, Yazoume Ye, Fred Arnold

ICF International, Calverton, MD, United States

Assessing the success of malaria control interventions is difficult due to the challenge of measuring malaria-specific outcomes. In contrast, hemoglobin levels are relatively simple to measure, and although not highly specific to malaria, moderate-to-severe anemia (hemoglobin < 8 g/dL) is a common symptom of the disease. Approximately 15% of anemia in pre-school-age children is attributable to malaria. To investigate the utility of moderate-to-severe anemia as an impact measure of malaria control interventions we analyzed data from 11 countries that had two or more demographic health surveys or malaria indicator surveys conducted between 2001 and 2011 containing information on insecticide-treated net (ITN) use and hemoglobin levels in children 6-23 months of age. Multivariate Oaxaca-Blinder decomposition for nonlinear response models with deviation contrast normalization for categorical variables were used to estimate the proportion of the decline in anemia prevalence over time due to increases in ITN use. Weighted average ITN use increased from 12.2% in baseline surveys to 44.3% in endline surveys and moderate-to-severe anemia decreased from 17.9% to 12.1%. In pooled, multi-country logistic regression models controlling for residence, household wealth, multiple birth status, mother's education, child's age and sex, and history of recent fever, odds of anemia were significantly lower for children who used an ITN the night before interview compared to those who did not (OR = 0.81, 95% CI = 0.70-0.94). Decomposition models reveal that the increase in ITN use between baseline and endline surveys accounted for 19% of the observed decrease in moderate-to-severe anemia which is equivalent to a 1.1% reduction in anemia prevalence. The changes in ITN use explained the greatest proportion of the total change in anemia between baseline and endline as compared to other covariates. Results suggest that scale-up of malaria control interventions are likely to have measurable impact on moderate-to-severe anemia and consequently that anemia may be a useful impact measure.

864

APPLICATION OF A *PLASMODIUM FALCIPARUM* WHOLE GENOME SNP MICROARRAY TO FIELD SAMPLES COLLECTED AS DRIED BLOOD SPOTS FROM SOUTHEAST ASIA

Christopher G. Jacob[1], John C. Tan[2], Becky A. Miller[2], Asako Tan[2], David Saunders[3], Charlotte Lanteri[3], Chanthap Lon[3], Rungniran Sug-arum[4], Thirasak Hoonchaiyaphum[4], Wichai Satimai[4], Toshihiro Mita[5], Mohammad Shafiul Alam[6], Jasmin Akter[6], Huang Fang[7], Kay Thwe Han[8], Shannon Takala-Harrison[1], Michael T. Ferdig[2], Christopher V. Plowe[1]

[1]Howard Hughes Medical Institute/University of Maryland School of Medicine, Baltimore, MD, United States, [2]Eck Institute for Global Health, Department of Biological Sciences, University of Notre Dame, Notre Dame, IN, United States, [3]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [4]Bureau of Vector Borne Diseases, Department of Disease Control, Ministry of Public Health, Nonthaburi, Thailand, [5]Department of Molecular and Cellular Parasitology, Juntendo University, Tokyo, Japan, [6]International Centre for Diarrhoeal Disease Research, Mohakhali, Dhaka, Bangladesh, [7]National Institute of Parasitic Diseases, Chinese Centre for Disease Control and Prevention, Shanghai, China, [8]Department of Medical Research (Lower Myanmar), Yangon, Myanmar

Genomic epidemiology studies of *Plasmodium falciparum* provide insights into parasite population structure, gene flow, drug resistance and vaccine development. In areas with adequate cold chain facilities, large volumes of leukocyte-depleted patient blood may be frozen for use in parasite genomic analyses. In more remote endemic areas, dried blood spot samples can be collected and stored at room temperature. The small volume of blood preserved on dried blood spots limits DNA yield and may preclude whole-genome sequencing. Analysis of single nucleotide polymorphisms (SNPs) allows for rapid evaluation of genome-wide diversity. Here we describe a DNA microarray that was designed for use on low DNA field samples to type a genome-wide set of SNPs that prior sequencing had shown to be variable in Africa, Southeast Asia, and Papua New Guinea. Our microarray uses variable length cDNA probes to type 33,728 genomic *loci* in the *P. falciparum* genome. We measured SNP calling accuracy by hybridizing purified DNA from sequenced malaria lab strains, and consistently achieved >95% accuracy in these samples. We then tested field samples with significantly lower DNA concentrations extracted from dried blood spots collected in therapeutic efficacy studies and routine surveillance studies in Bangladesh, Cambodia, China, Myanmar, and Thailand. Our new high-density microarray provided high SNP call rates from a wide range of parasite DNA quantities (~2-250ng) determined by 18s qPCR extracted from whole blood and dried blood. Field samples with parasite DNA quantities below 2ng were whole-genome amplified before running on the microarray. The microarray assay performed adequately on dried blood spots, and may provide a useful tool for genome-wide analysis of malaria parasites in diverse settings.

865

THE BURDEN OF *GAMETOCYTEMIA* AND PLACENTAL MALARIA IN PREGNANT WOMEN IN BLANTYRE, MALAWI

Sarah Boudova[1], Lauren Cohee[1], Linda Kalilani-Phiri[2], Phillip Thesing[3], Mwayi Madanitsa[2], Jenny Walldorf[1], Steve Kamiza[2], Atis Muehlenbachs[4], Terrie E. Taylor[5], Miriam K. Laufer[1]

[1]University of Maryland, Baltimore, MD, United States, [2]University of Malawi, Blantyre, Malawi, [3]Blantyre Malaria Project, Blantyre, Malawi, [4]University of Washington School of Medicine, Seattle, WA, United States, [5]Michigan State University, East Lansing, MI, United States

Women living in malaria-endemic areas, semi-immune to malaria normally, become newly susceptible to malaria infection during pregnancy. The goal of this study was to assess the extent to which pregnant women serve as a reservoir of malaria gametocytes, and thereby a source of continued transmission. We hypothesized that pregnant women may have significant *gametocytemia* due to chronic placental sequestration. We used specimens from an observational study of malaria during pregnancy conducted in Blantyre, Malawi in 2009-10. All women received sulfadoxine-pyrimethamine as intermittent preventive treatment and all episodes of *parasitemia* were treated. We performed light microscopy on all positive malaria smears to detect gametocytes. Placental samples were tested for active malaria infection by quantitative PCR and for past infection by histological examination for hemozoin pigment. Proportions were compared by Fisher's exact test. 2742 blood smears from 450 women were examined for evidence of malaria. Among these women, 79 (17.6%) were positive for asexual stages at least once, and among that subset, 11 (13.9%) were positive for gametocytes. In gametocyte-positive smears, the average gametocyte density was 70/µL blood. Placental specimens were available for 321 deliveries. *Gametocytemia* was strongly associated with active placental malaria (p=0.003), and past infection (p<0.001). All women with gametocytes detected in a peripheral blood smear had hemozoin detected in the placenta. The proportion of pregnant women with malaria infection who have microscopically detectable gametocytes is higher than is usually detected among adults in neighboring countries. Because malaria infection is frequent in the pregnant population and women are generally asymptomatic at the time of infection, pregnant women may serve as important reservoirs of transmission in malaria-endemic areas and this population may warrant specific attention in malaria elimination efforts.

866

AN UPDATE ON THE POTENTIAL FOR NORTH AMERICAN MOSQUITOES TO TRANSMIT RIFT VALLEY FEVER VIRUS

Michael J. Turell[1], Seth C. Britch[2], Robert C. Aldridge[2], Brian D. Byrd[3], Bruce A. Harrison[3], Kenneth J. Linthicum[2]

[1]United States Army Medical Research Institute for Infectious Diseases, Fort Detrick, MD, United States, [2]United States Department of Agriculture-Center for Medical, Agricultural and Veterinary Entomology, Gainesville, FL, United States, [3]Western Carolina University, Cullowhee, NC, United States

Rift Valley fever virus (RVFV) poses a serious threat to both North America agriculture and to human health. As illustrated with the introduction and spread of West Nile virus, exotic pathogens have the potential to cause significant economic damage as well as illness in North America. Despite its potential to cause devastating injury to cattle, goats, and sheep, little is known about the potential for North American mosquitoes to transmit RVFV, and this information is needed to develop an appropriate response to the introduction of RVFV. Therefore, we have been evaluating the potential for a variety of North American mosquitoes to transmit this virus either horizontally or vertically. Data on the ability of >20 common North American mosquito species to become infected with and to transmit RVFV will be presented, including the ability of at least five species, representing two genera (*Psorophora* and *Mansonia*), for which there are no published data. While *Culex tarsalis*, *Aedes japonicus*, and *Psorophora ferox* were among the most competent laboratory vectors for RVFV, *Cx. quinquefasciatus*, *Cx. nigripalpus*, and *Anopheles crucians* were virtually incompetent, even when fed on hamsters with viremias $>10^{9.5}$ plaque-forming units (PFU)/ml. Although *Ps. columbiae* was among the most susceptible to oral infection, and most specimens tested developed a disseminated infection, there was a significant salivary gland barrier and none of the members of this species transmitted RVFV by bite, including those inoculated intrathoracically. In addition to laboratory vector competence, factors such as seasonal density, host feeding preference, longevity, and foraging behavior should be considered when determining the potential role that these species could play in RVFV transmission.

867

BLOOD-MEAL SOURCE INFLUENCES THE INTERACTION BETWEEN THE MALARIA VECTOR *ANOPHELES GAMBIAE* AND THE MALARIA PARASITE *PLASMODIUM FALCIPARUM*

Amélie Vantaux, Anna Cohuet, Thierry Lefèvre

Institut de Recherche pour le Développement, Montpellier, France

Although it is widely accepted that *Anopheles gambiae* displays an extreme form of specialization for human hosts as a source of blood, it can also feed on a wide range of other vertebrate hosts. Recent studies have addressed the consequences of feeding on different vertebrate hosts on the fitness of mosquito vectors. However, it is currently unknown whether different blood-meal sources can also impact the development of malaria parasites within the mosquito vectors Here we explored the effects of a range of blood-meal sources on *An. gambiae-Plasmodium falciparum* interactions. Following an infectious blood-meal, mosquitoes were fed with blood from either chicken, cow, human, or sheep for the next 3 gonotrophic cycles. We then measured various fitness-related traits including mosquito fecundity, daily mortality rate and progeny development as well as parasite prevalence and intensity. Mosquito survival was strongly impacted by the blood-meal type, with avian blood substantially reducing mosquito survival. Compared to uninfected individuals, infected mosquitoes displayed reduced lifespan. Finally, we provide the first evidence that mosquito blood-meal source can modify the development of malaria parasites. Together, our data demonstrate that blood-meal source can shape *An. gambiae -P. falciparum* interactions and has ultimately the potential to influence the dynamic of malaria transmission.

868

EAVE CURTAINS: ENTOMOLOGICAL EVALUATION AND COMMUNITY KNOWLEDGE, ATTITUDES AND PERCEPTIONS IN PREVENTION OF MALARIA MOSQUITO ENTRY INTO HUMAN DWELLINGS IN KISIAN AND ROTA VILLAGES, KISUMU COUNTY, WESTERN KENYA

Bernard O. Abong'o

KEMRI/Centers for Disease Control and Prevention - Kenya, Kisumu, Kenya

Vector control has overwhelmingly relied on insecticides that target the adult stage of mosquitoes but with very scant consideration of simple changes in house designs that have potential of protecting people against malaria. Several studies have demonstrated that house modification with screens and ceilings reduces entry of mosquitoes into human dwellings, presumably reducing malaria transmissions. However community knowledge, attitudes and perceptions of such a tool remain unclear. Besides, community implementation of improved house designs is lacking. In addition, house modification may be countered with behavioral modifications from vectors that are exquisitely adapted to feeding and resting indoors. The purpose of this study will be to assess community knowledge, perceptions and attitudes towards house modification for vector control and to investigate the impact of eave screening on entomological indicators and changes in malaria care seeking behavior of individuals. The study will be conducted in Kisian and Rota villages in Kisumu county, western Kenya. One hundred and twenty pairs of neighboring houses with similar structural designs for each pair will be selected. For all the selected houses, a questionnaire assessing knowledge, attitude and perception of house screening for malaria control will be administered to household heads. In each pair, the houses will be randomly allocated to either receive untreated eave curtain or no eave curtain, controls. The intervention houses will be screened at the beginning of the study while control houses will be screened at the end of the study. Four rounds of sampling will be done fortnightly before screening and for three months thereafter by mechanical aspiration. The sampled mosquitoes will be identified both morphologically and by polymerase chain reaction (PCR), while female *Anopheles* will be tested for the presence of sporozoites and abdomens of fed and half gravid females will be analyzed for host blood meal detection. Results will be expected to show the community attitude towards house modification and possible behavioral modification by *Anopheles* mosquitoes due to changes in house design which will be critical for further vector control efforts.

869

DISENTANGLING THE IMPACTS OF SEASONALITY, TEMPERATURE AND LAND COVER ON THE ABUNDANCE OF ARBOVIRAL MOSQUITO VECTORS IN CALIFORNIA USING GENERALIZED ADDITIVE MODELS

Robert C. Reiner[1], David M. Hartley[2], Tianchan Niu[2], T. Alex Perkins[1], Nakul Chitnis[3], Donal Bisanzio[4], William K. Reisen[1], Christopher M. Barker[1]

[1]University of California Davis, Davis, CA, United States, [2]Georgetown University, Washington, DC, United States, [3]Swiss Tropical and Public Health Institute, Davis, Switzerland, [4]Emory University, Atlanta, GA, United States

Mathematical models for the transmission of mosquito-borne diseases often rely on very simple assumptions about the population dynamics of the mosquito vectors. Linking such models to real-world trapping data on vector abundance requires a method that smooths over the discontinuous trapping data to yield complete time series, simultaneously accounting for the effects of other key environmental variables that also vary in space or time. Generalized additive models (GAMs) offer a flexible way to disentangle the relative roles of seasonality, temperature, and land cover as predictors of mosquito abundance, and here, we consider their ability to explain the spatio-temporal abundance patterns of the key arboviral vectors, *Culex tarsalis*, the *Cx. pipiens* complex, and *Aedes*

melanimon. Models were fitted to a very large surveillance data set from California (2003-2009, 102,188 trap-nights of 4,882,911 mosquitoes), and results will be used to inform models for the transmission dynamics of West Nile virus and Rift Valley fever virus. Preliminary results indicate significant effects of all three drivers (P<0.001), as well as significant interaction effects between day of year and temperature (P<0.001). The seasonal signal, as well as overall magnitude, for different land cover types is significantly different and matches known observed dynamics such as late-season flooding of wetlands that produces a late boost in abundance near wetlands. These model results provide a baseline for mosquito abundance that can be used in both persistence and invasion models of arboviruses, and the smooth functions that make up the GAMs allow for reasonable low levels of extrapolation. For example, since we account for both asynoptic temperature fluctuations and differentiate across land-types, we can both predict abundances in scenarios where there are small systematic increases (or decreases) in temperature throughout the year as well as scenarios where land types change through either conservation or urbanization.

870

THE IMPACT OF CLIMATE ON WEST NILE VIRUS TRANSMISSION ACROSS NORTH AMERICA

A. Marm Kilpatrick[1], Noah S. Diffenbaugh[2], Dan Horton[2], Laura D. Kramer[3]

[1]University of California Santa Cruz, Santa Cruz, CA, United States, [2]Stanford University, Stanford, CA, United States, [3]Wadsworth Center, Slingerlands, NY, United States

In the fourteen years since West Nile virus arrived in North America, there has been enormous year to year variation. Three years, 2002, 2003, and 2012 had regional epidemics with more than 2500 neuroinvasive cases, 250 deaths, and many more febrile cases. In most other years there were relatively few cases. The causes of this year to year variation has not yet been identified, although climatic conditions have been invoked frequently. We present the results of an analysis of coarse continental county scale weekly data and detailed local scale to uncover the impact of climatic drivers. We find, somewhat surprisingly, that temperature and precipitation are not especially strong drivers of year to year variation, and the impact of temperature is nonlinear. These results highlight the importance of rigorous analyses of climate and vector borne disease, especially in the case of zoonotic pathogens where many factors can influence transmission to humans.

871

THE EFFECT OF DENGUE VIRUS INFECTION ON *AEDES AEGYPTI* RESPONSE TO DEET

Victor Sugiharto[1], John Grieco[1], Michael J. Turell[2], Edgar D. Rowton[3], Michelle G. Colacicco-Mayhugh[4], Nicole L. Achee[1]

[1]Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [2]Department of Vector Assessment, Virology Division, United States Army Medical Research Institute for Infectious Diseases, Fort Detrick, MD, United States, [3]Division of Entomology, Walter Reed Army Institute of Research, Silver Spring, MD, United States, [4]Preventive Health Services Department, Medical Education and Training Campus, Fort Sam Houston, San Antonio, TX, United States

Mosquito behaviors, such as host-seeking, landing, probing and biting, are driven by chemical signals within the environment that are recognized by the olfactory system. Because dengue virus (DENV) replicates and remains at elevated levels within the mosquito brain during DENV infection, this pathophysiology may interrupt behavioral responses to chemicals used in products for protection from biting insects, potentially reducing intervention efficacy. Three different *Aedes aegypti* treatment groups, DENV-1 injected, diluent-injected, and un-injected, were exposed to DEET (2.5% and 0.14%) at various days post-injection (1, 4, 7, 10, and 14 dpi), in a laboratory assay designed to measure contact irritancy behavior in mosquitoes. Our results indicate a significant escape response in all test populations when exposed to DEET and this behavior did not differ significantly among DENV-1 injected, diluent-injected and un-injected groups. This outcome suggests that DENV-1 infection in mosquitoes does not interfere with the underlying mechanisms that drive an irritancy response to DEET, and that insect repellents may represent a viable tool for preventing contact between humans and DENV-infected mosquitoes. Ongoing studies include measuring Ae. *aegypti* gene-regulation after infection with DENV-1.

872

COMPARISON OF DENGUE VIRUS VECTOR COMPETENCE OF *AEDES MEDIOVITTATUS*, THE CARIBBEAN TREEHOLE MOSQUITO AND *AEDES AEGYPTI*

B. K. Poole-Smith[1], Ryan R. Hemme[1], Mark Delorey[2], Andrea L. Gonzalez[1], Manuel Amador[1], Gilberto Felix[1], Roberto Barrera[1], Elizabeth A. Hunsperger[1]

[1]Centers for Disease Control and Prevention, San Juan, PR, United States, [2]Centers for Disease Control and Prevention, Fort Collins, CO, United States

Aedes mediovittatus is found throughout the Caribbean and often shares habitat with *Aedes aegypti*. After intensive vector control measures targeted the removal of *Ae. aegypti* in Cuba, it was reported that *Ae. mediovittatus* was able to invade and exploit these same artificial habitats. Vector control measures specific to *Ae. aegypti* in Puerto Rico may create a habitat vacancy that *Ae. mediovittatus* could exploit. A previous study showed that *Ae. mediovittatus,* as well as *Ae. aegypti,* can be infected by DENV-1 (PR-1318) and *Ae. mediovittatus* was more susceptible to infection with DENV-2 (New Guinea C, PR-1328) than *Ae. aegypti*. However DENV-3 and DENV-4 infection competence remains unknown. To determine the vector competence of *Ae. mediovittatus* for DENV 1-4, mosquitoes were infected *per os* with DENV-1 (HAW), DENV-2 (New Guinea C), DENV-3 (H87), DENV-4 (H241) at titers of 5-6 logs plaque-forming unit (pfu) equivalents. At 14 days post infection, samples were collected, RNA extracted, and tested by qRT-PCR to determine infection and transmission rates; results were standardized to pfu equivalents. A generalized linear model assuming a binomial distribution and using a log link was used to analyze infection rates. Infection rates between *Ae. aegypti* and *Ae. mediovittatus* are DENV-1 (15.0% vs. 13.3%), DENV-2 (16.7% vs. 25%), DENV-3 (10.0% vs. 18.3%) and DENV-4 (61.7% vs. 1.7%). Transmission rates between *Ae. aegypti* and *Ae. mediovittatus* are DENV-1 (6.7% vs. 11.7%), DENV-2 (1.7% vs. 1.7%), DENV-3 (5.0% vs. 6.7%) and DENV -4 (41.7% vs. 1.7%). Infection and transmission rates of the two species did not statistically differ for DENV-1, DENV-2, or DENV-3. *Ae. aegypti* had statistically higher infection and transmission rates for DENV-4. Vector control measures for dengue prevention in the Caribbean may need to account for *Ae. mediovittatus* which may be capable of transmitting DENV in the absence of *Ae. aegypti*. Future studies of *Ae. mediovittatus* will evaluate competence for recent dengue virus isolates.

873

MOSQUITO MAGNET® LIBERTY PLUS TRAP BAITED WITH OCTENOL CONFIRMED BEST CANDIDATE FOR *ANOPHELES* SURVEILLANCE AND PROVED USEFUL IN PREDICTING THE RISK OF MALARIA TRANSMISSION IN FRENCH GUIANA

Samuel Vezenegho[1], Pascal Gaborit[1], Romuald Carinci[1], Jean Issaly[1], Isabelle Dusfour[1], Sébastien Briolant[2], Romain Girod[1]

[1]Institut Pasteur de la Guyane, Cayenne, French Guiana, [2]Institut de Recherche Biomédicale des Armées/Institut Pasteur de la Guyane, Cayenne, French Guiana

Malaria is a major public health problem in French Guiana. In the context to establish a policy to control and eliminate malaria, monitoring systems for anopheles populations that can contribute to predict epidemic risk and

to set priorities for malaria vector control are critical. Sampling *Anopheles* by the gold standard human landing catch (HLC) method faces ethical constraints. Alternative traps are available but their efficacy varies and depends on mosquito behaviors. *Anopheles darlingi*, the main vector in French Guiana, bites and rest outdoors in inland Amerindian villages. We first evaluated the efficacy of Mosquito Magnet® trap baited with Lurex3™ (MM1) or octenol (MM2) and double mosquito net (DMN) baited with human against HLC for sampling outdoor anopheles. Association between anopheles densities collected with the most efficient trap and HLC was then investigated in a year study. Finally, the selected trap was used to collect entomological data and analyzed for association with clinical malaria cases after a six-month study aiming at predicting the risk of contracting malaria. In all, 361 anopheles were collected during traps evaluation and only sampling by MM2 showed no statistically difference with HLC (p=0.1178). Of the 325 *An. darlingi* captured, MM1 contributed 9%, MM2 29% and HLC 62%. Under the year long study, MM2 and HLC showed strong association (p<0.001, Spearman's coefficient: 0.7280) when analyzed for An. *darlingi* collections (n= 5726). Under the six-month study, 216 An. *darlingi* were collected with MM2 and 136 malaria cases were reported by the local health center. A significant correlation (p=0.0003; Spearman's r = 0.6494) between An. *darlingi* density and clinical malaria cases was noted. MM2 demonstrated potentials as a replacement for HLC in providing useful information needed for *Anopheles* surveillance in view to implement an efficient and sustainable malaria control strategy. Further assessment of MM2 for anopheles collections in French Guiana and modeling its data for predicting human biting rate is recommended.

874

ENTEROBACTER SP. IN THE MOSQUITO GUT: LPS DEFICIENCY AFFECTS GUT COLONIZATION AND MALARIA SUSCEPTIBILITY IN *ANOPHELES GAMBIAE*

Jinjin Jiang, Wanqin Yu, Dong Pei, Phanidhar Kukutla, Jiannong Xu

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

The mosquito gut ecosystem harbors a complex microbial community, which varies across life stages. Mosquitoes take blood meals for egg production. Blood feeding enables disease transmission. Blood meals can significantly reduce the community diversity and favor enteric bacteria. *Enterobacter sp.* is a dominant bacterium that expands after blood feeding in the gut of the mosquito *Anopheles gambiae*. Therefore Enterobacter could be a good model bacterium to study bacterial behavior in the blood fed gut. We isolated a strain of *Enterobacter sp.* and sequenced its genome. To be able to study genes that are involved in the colonization of the gut, we conducted mutagenesis in *Enterobacter* and generated a mutant library using EZ-Tn5™ Tnp Transposome Kit (Epicentre). Three mutants were identified, in which alpha1,2-glucosyltransferase (waaJ), O-antigen ligase (waaL), LPS heptosyl transferase I (waaC), were disrupted by insertion, respectively. These genes are clustered in the same genomic region, and all involved in LPS biosynthesis. These mutants have deficient LPS structure, as shown in the PAGE gel. In addition, the mutants are much more sensitive to paraquat, an strong oxidative stress inducer, compared to the wild type. We tagged wild type and waaL mutant with GFP or RFP, respectively. Tagged bacteria were traceable after introducing into the gut via oral feeding. Tagged wild type (wt) bacteria well proliferated after blood feeding, and excreted in the feces around 48hr post feeding. However, the capacity of gut colonization was compromised in the LPS deficient mutants. In addition, the LPS deficiency also affected mosquito susceptibility to malaria. When infected with malaria *Plasmodium berghei* the mosquitoes with wt bacterial reconstruction and unmanipulated control mosquitoes had similar infection pattern, while mosquitoes with mutants had significant higher oocyst load. The data suggest that bacterial LPS structure is important for the gut colonization. The investigation of the effects of LPS deficient mutants on the gut basal immunity is under way.

875

TRANSMISSION OF DENGUE HEMORRHAGIC FEVER IN ITS RELATIONSHIP BY CLIMATE VARIABILITY IN JAKARTA, INDONESIA

Margareta Maria Sintorini, Hilarion Widyatmoko Widyatmoko

Trisakti University, Jakarta Barat, Indonesia

Dengue hemorrhagic fever (DHF) has become endemic in many big cities in Indonesia. The Indonesian geography, the population growth, the climate change, the low level awareness and knowledge have caused the DHF taking place, which more over tending to increase in number. The objective of this research is to identify the transmission dynamic of DHF cases related to the pattern of the climate variability in Jakarta. The study was conducted in 5 sub district from April 2012 until March 2013. This research uses the design of ecological study with hypothesis test model and statistical analysis. Respondents of 844 households were interviewed to explore their knowledge, attitude and practice (KAP) regarding DHF using a standard questionnaire. The result indicate, that the DHF cases in Jakarta are influenced by precipitation (0.000), temperature ambient (0.000), indoor humidity (0.003), outdoor humidity (0.000), Man Landing Rate *Aedes* (0.016), resting habit *Aedes* (0.000) and community knowledge (0.008). The most influential climate factors to the DHF cases are precipitation, temperature, humidity and the low level of the community knowledge. The simulation model indicates a factor that may decrease DHF was mosquito larva monitoring and knowledge enhanced.

876

ESTIMATING THE HERITABILITY OF EXTRINSIC INCUBATION PERIOD IN DENGUE INFECTED MOSQUITOES

Yixin Henry Ye[1], Francesca D. Frentiu[2], Nigel W. Beebe[3], Andrew van den Hurk[4], Scott L. O'Neill[1], Stephen F. Chenoweth[3], Elizabeth F. McGraw[1]

[1]Monash University, Melbourne, Australia, [2]Queensland University of Technology, Brisbane, Australia, [3]University of Queensland, Brisbane, Australia, [4]Queensland Health, Brisbane, Australia

In the past 20 years, dengue has re-emerged to become the most prevalent arthropod-borne virus affecting humans today. This exponential increase in disease incidence has brought with it significant health, social and economic problems. Dengue is most commonly vectored by the mosquito *Aedes aegypti*. Vectorial capacity, which is a measurement of the efficiency of vector-borne disease transmission, is influenced by a few key factors. Extrinsic incubation period (EIP), which is the interval of time from the ingestation of an infectious bloodmeal to the time of transmission, is a key determinant of vectorial capacity. Previous studies have estimated the heritability of susceptibility of *Ae. aegypti* to dengue and used quantitative genetic techniques to identify *loci* that underpin this trait. The genetic basis of EIP in mosquitoes is not well understood largely due to the technical difficulty in measuring this trait. Here we have carried out a quantitative genetic study using a full-sib breeding design to estimate the heritability of EIP in dengue infected *Ae. aegypti*. Using a non-destructive method to measure EIP we have also been able estimate heritability of body size, susceptibility to dengue infection and viral titer in the same mosquitoes. The heritabilities and correlations between these four traits reveal the genetic architecture of vector competence and the evolutionary potential for EIP.

877

MOSQUITO SALIVA FACILITATES CHIKUNGUNYA VIRUS INFECTION AND DISSEMINATION

Saravanan Thangamani[1], Meghan E. Hermance[1], Stephen Higgs[2]

[1]University of Texas Medical Branch, Galveston, TX, United States, [2]Kansas State University, Manhattan, KS, United States

Chikungunya virus (CHIKV) is an alphavirus belonging to the Togaviridae family that is transmitted to humans by infected mosquitoes. The virus is delivered to humans together with mosquito saliva at the bite site. The complex repertoire of secreted bioactive molecules in mosquito saliva can have profound effects on transmission efficiency, pathogen establishment, and disease pathogenesis. In this work, we have investigated the role of mosquito saliva to facilitate CHIKV transmission and establishment of infection. Three groups of mice were intra-dermally inoculated with a) Salivary gland extracts (SGE), b)CHIKV+SGE, c) CHIKV alone. were intra-dermally delivered to mice ear with and without CHIKV. Skin biopsy at the site of infection (ear pinna), blood, tissues (auricular lymph nodes, spleen, liver, brain, skeletal muscle), and limbs were collected at 48, 72 and 96 hours post-infection (hpi) for comparative histological and molecular analysis. In skin, IL-1β, IL-6, IFN-γ, CCR1, CCL7, CCL2 and TNF-α were significantly upregulated in CHIKV+SGE injected animals compared with that of CHIKV alone. This suggest a potent innate-like immune response dominated by macrophages and neutrophils. In the lymph nodes, IL-12, IFN-γ, CCL7, CCL-2, CXCL5 were significantly upregulated in CHIKV+SGE injected animals compared with that of CHIKV alone. In muscle, IL-12, IFN-γ, TNF-α, CXCL5, CCL7, CCR1 were upregulated. In the early time points of this study, a mixed immune response was observed. Both IL-12 and IFN-γ were significantly upregulated after 72 hpi suggesting a Th1 type immune response at the later stage. Overall, pro-inflammatory cytokines/chemokines from the skin, lymph node and muscle were upregulated. CHIKV was detected in skin, muscle, lymph node and spleen samples, but not in brain and liver samples. Inclusion of SGE with CHIKV resulted in enhanced/elevated viremia in skin, lymph nodes and muscle of animals compared to that of CHIKV alone. These data clearly show that Ae. *aegypti* SGE enhances the key process of early CHIKV infection and dissemination to lymph nodes and muscle.

878

VECTOR COMPETENCE OF *CULEX PIPIENS QUINQUEFASCIATUS* (*DIPTERA: CULICIDAE*) FOR WEST NILE VIRUS ISOLATES FROM FLORIDA

Stephanie L. Richards[1], Sheri L. Anderson[2], Cynthia C. Lord[2]

[1]East Carolina University, Greenville, NC, United States, [2]University of Florida, Vero Beach, FL, United States

Culex pipiens quinquefasciatus is a vector of West Nile virus (WNV) in the United States. Four WNV isolates (WN-FL-03, WN-FL-05-558, WN-FL-05-2186, and WN-FL-05-510) collected from different regions of Florida (FL) were used for vector competence experiments in FL *Cx. p. quinquefasciatus.* Vector competence was compared between two mosquito colonies for WN-FL-03. Mosquitoes were fed blood containing 7.9±0.2 $\log_{10}$ plaque-forming units WNV/mL ± SE and incubated at 28°C for 14d. Vector competence was evaluated (χ^2,P<0.05) for rates of infection, dissemination, and transmission and virus titer in bodies, legs, and saliva was compared (ANOVA, P<0.05). Infection and dissemination rates (≥ 95%) were not affected by isolate or colony. Transmission of WNV (0-20%) was affected by isolate and colony and was observed only in mosquitoes fed WN-FL-05-558 and WN-FL-05-2186. Significant differences were observed between isolates and colonies for WNV titers of body and leg tissues, but not saliva. The highest body titers were observed in mosquitoes fed WN-FL-05-558 and the highest leg titers were observed in those fed WN-FL-05-558 or WN-FL-05-2186. High leg titers in the group fed WN-FL-05-558 or WN-FL-05-2186 were associated with transmission. *In vitro* growth rate in Vero cells was evaluated for WNV isolates. Supernatant was sampled at 24h, 48h, 72h, 96h, 120h, 144h, and 168h post-inoculation. Viral titers in WN-FL-03 were significantly higher (ANOVA, P<0.05) than other isolates from 24h–144h except for WN-FL-05-558 that was highest at 120h. No titer differences were observed between isolates at 168h. Variation in vector competence and *in vitro* growth rates between isolates may be related to genetic differences shown by a previous study. Low transmission rates suggest that isolates were affected differently by the salivary gland transmission barrier. Geographic variation in vector competence of mosquitoes may be attributed, in part, to their exposures to WNV isolates with different growth rates. The evaluation of differences in vector competence and *in vitro* virus growth kinetics for WNV isolates from different regions may help us understand vector-virus interactions and, hence, the role of vectors in virus transmission cycles in nature.

879

AEDES ALBOPICTUS AS MAIN VECTOR OF FIRST DENGUE HEMORRHAGIC FEVER OUTBREAK IN KAIMANA DISTRICT WEST PAPUA: AN ENTOMOLOGY SURVEY AND MOLECULAR APPROACH

Hana Krismawati, Tri Nury Kridaningsih, Antonius Oktavian, Evy Iriani Natalia, Mardi Rahardjo

Institute of Research and Development for Biomedicine Papua, Jayapura-Papua, Indonesia

Kaimana District was an coastal area in West Papua (0-100 above sea level). Dengue outbreak first case 50 years had been occured in May-July, presumptively caused by transmission of Dengue virus from infected temporer visitor that participating one of religious event, held by local goverment. Entomological survey was intended to identified the species, breeding habitat of suspected mosquito vectors and detection of the present of virus in mosquitoes as well as in larvae. Investigation were overtaken on Juny 2012. Mosquitoes sample were collected on resting habitat as well as human landing collection. Larvae were collected from water container at 25 sampling site consist of patient household, school, and hospital. Larvae collection was rearranged in Entomology Laboratory. Dengue virus were detected in mosquitoes that had been collected from research area as well as from rearranged mosquitoes using RT-PCR method Lancioti primer. Case report show that the age majority of DHF cases is 6-12 (48,1%) and dominan patients were woman (63%). The study found one dead (3,7%) cases and 26 (96,3%) sick cases. Result of entomology survey indicated that *A.agyepti* had been known as dengue main vector in most area in Indonesia were absent, while the second vector *A.albopictus* was present abundantly in adult stage as well as in larvae stage that had been collected. The mosquitoes were found more in their resting habitat than in patient's houses. The most abundant of the mosquitoes was found at 13.00 in SDN Kaimana one of MTQ event took place. The container that contain *A.albopictus* larvae were unused tire, plastic container and ceramic container. The study from 25 sampling location indicated *House Indek* 26,6% and *Container Indek* 21,2% . The house index was more than 10% mean that this area has high susceptibility in DHF outbreak according to Ministry of Health criteria. Molecular detection of virus on samples showed that the dens virus was been detected on mosquitoes as well as on larvae.

880

MALARIA RISK AMONG FISHERMEN AND SUBSISTENCE FARMERS OF RUSINGA ISLAND, WESTERN KENYA

Evelyn A. Olanga[1], Lucy Irungu[2], Richard Mukabana[1]

[1]International Centre of Insect Physiology and Ecology, Nairobi, Kenya, [2]University of Nairobi, Nairobi, Kenya

Malaria is a leading cause of morbidity and mortality in Kenya. The highest burden of malaria occurs in the western part of the country where transmission occurs throughout the year. Evidence suggests that malaria transmission is unevenly distributed between individuals in populations. Some people are at a higher risk of infection than others. Although

malaria transmission is higher in western Kenya than other areas of the country, there are factors that influence the risk of transmission, such as peoples' behavior patterns, environmental factors, and socio-economic status. A cross-sectional study was conducted in western Kenya to determine factors linked to livelihood-related activities that influence malaria risk. A total of 248 fishermen and 114 farmers were randomly recruited into the study. House to house visits were conducted whereby participants were interviewed using a structured questionnaire and blood test for malaria infection administered. The variables that were studied included location of residence (zone), mode of dress while carrying out livelihood activities, housing characteristics, time of work, and use of malaria protection measures while working. The relationship between variables and malaria were analyzed by fitting a generalized linear model (GLM). Fishermen and farmers living in two zones were six times more likely to be infected with malaria than their counterparts in the other six zones. Malaria infection was significantly higher in individuals who dressed scantily (clothes that exposed their arms and legs) while working outdoors than among counterparts who were fully dressed. Individuals who worked at night were more likely to suffer from malaria than those who worked during the day. Fishermen and farmers both work at odd hours during peak biting times of malaria-transmitting mosquitoes, that is, during twilight zones and at night. In conclusion, location of residence, mode of dressing while at work and time of work were prominent risk factors for malaria infection among fishermen and farmers of Rusinga Island.

881

AUTODESSIMINATION OF PYRIPROXYFEN TO *ANOPHELES ARABIENSIS* BREEDING HABITATS: A NOVEL LARVICIDING APPROACH

Dickson W. Lwetoijera[1], Caroline Harris[1], Stefan Dongus[1], Gregor Devine[2], Philip McCall[3], Silas Majambere[1]

[1]Ifakara Health Institute, Ifakara, Morogoro, United Republic of Tanzania, [2]Tropical Public Health Services - Cairns, Queensland, Australia, [3]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

While conventional larviciding could greatly complement current malaria vector control interventions, its high operational cost and the difficulty in locating all mosquito-breeding habitats in rural settings reduces its effectiveness. Autodissemination of larvicides by mosquitoes offers a new possibility for more effective larviciding. This study assessed the potential for *Anopheles arabiensis* to transfer pyriproxyfen (PPF), a potent larvicide, from contamination sites to their breeding habitats. Different mechanisms for contaminating An. *arabiensis* were tested inside separate sections of a semi field system (SFS) in rural Tanzania. We assessed whether a cow, clay pots, or cow shed roofs and walls sprinkled with PPF powder, could contaminate mosquitoes while blood feeding or resting. Treatment was done using 10% AI PPF powder, and a control was left untreated. In each SFS section, a cow shelter (mud hut) was built, clay pots were provided as resting sites and artificial breeding habitats were created. Unfed adult female An. *arabiensis* were released inside the SFS and a cow was provided for blood meal. Egg and larval presence were monitored daily from the breeding habitats provided, and pupae moved into cages to allow for exact emergence rates to be calculated. Using a treated cow, mosquitoes were contaminated with PPF during blood feeding and larval bioassays using these mosquitoes demonstrated a significant adult emergence inhibition, proving that mosquitoes were able to pick up PPF. With clay pots, mosquitoes were contaminated while resting and a 90% adult emergence inhibition was observed in breeding habitats in the same section, suggesting that PPF had been disseminated by the contaminated mosquitoes. Similarly, treating the cow shed successfully contaminated resting mosquitoes and completely prevented them from laying eggs. These findings indicate the possibility of auto-dissemination of PPF by *An. arabiensis* but also the sterilization of adult mosquitoes with this insecticide.

882

THE ROLE OF CD8+ T CELLS IN PATHOLOGICAL GIARDIASIS

Aleksander Keselman, Steven M. Singer

Georgetown University, Washington, DC, United States

Infection with the protozoan *Giardia duodenalis* is the most common intestinal parasitic disease in the United States. Disease transmission occurs with consumption of cyst-contaminated food or water. Clinical giardiasis presents with diarrhea and malabsorption of nutrients. Animal models have been helpful in defining the molecular processes that impair nutrient absorption and induce osmotic shifts within the infected gut to cause diarrheal disease. One major pathological hallmark of *Giardia* infection is the reduction of intestinal digestive enzymes, such as disaccharidases. β2m-/- mice, that lack functional CD8+ T cells, do not exhibit reduced disaccharidases and yet clear infection with normal kinetics. Disaccharidase reduction has also been recapitulated in athymic mice receiving CD8+ T cells from infected donor mice by adoptive transfer. This suggests that CD8+ T cell responses during *Giardia* infection are strictly pathogenic. In light of these observations, we have devoted our efforts to understanding how G. duodenalis infection activates intestinal CD8+ T cells to drive pathology. We report an increase of CD8+ T cells within the duodenum of infected mice. Analysis of surface marker expression on intestinal CD8+ T cells revealed an increased CD44hi population following infection within the lamina propria. These CD44hi cells produced IFNγ and TNFα. *Giardia* is non-invasive and persists within the intestinal mucosa without breaching the epithelial barrier or infecting host cells directly. Therefore, it is interesting that a CD8+ T cell response is triggered and that this response does not confer protection but rather leads to intestinal injury. The dependence on antigen in this response is currently being pursued in order to determine if *Giardia*-activated CD8+ T cells are mounting a specific response or are acting as bystanders. Further characterization of these intestinal CD8+ T cell responses to *G. duodenalis* infection will expand our understanding of pathogenesis and help identify novel therapeutic targets for the clinical setting.

883

PRIMARY AMEBIC MENINGOENCEPHALITIS ASSOCIATED WITH THE PRACTICE OF RITUAL NASAL RINSING - ST. THOMAS, U.S. VIRGIN ISLANDS, 2012

Jamae Morris[1], Azizeh Nuriddin[1], Tai Hunte[2], Thomas Morris[2], Vincent Hill[1], Bonnie Mull[1], Lihua Xiao[1], Dawn Roellig[1], Alexandre da Silva[1], Julia Gargano[1], Jonathan Yoder[1], Michael Beach[1], Jennifer Cope[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Virgin Islands Department of Health, St. Thomas, U.S. Virgin Islands

Primary amebic meningoencephalitis (PAM), an almost universally fatal condition, affects 0-8 persons annually in the U.S. PAM results when *Naegleria fowleri*, a free-living ameba found in warm freshwater, enters the nose and migrates to the brain. While most infections are associated with recreational freshwater exposure, nasal rinsing has recently emerged as a mode of transmission. On November 21, 2012, the U.S. Virgin Islands (USVI) Department of Health documented the first PAM infection and death in the territory. Infection occurred in a 47-year-old Muslim male who practiced ablution, a required ritual cleansing done in preparation for prayer that sometimes involves nasal rinsing. An investigation was conducted to characterize the patient's water exposures and describe community ablution practices. In December 2012, semi-structured interviews were conducted with the patient's roommate and a snowball sample of participating members of his mosque. Environmental investigations, including water sampling, were conducted concurrently at the case-patient's home and mosque. The patient had no recreational freshwater exposure and primarily practiced ablution at home using untreated rainwater and groundwater and at the mosque using municipal water. All 22 participants practiced ritual ablution; 86% performed nasal rinsing. To be acceptable for ablution, water must simply look, smell,

and taste clean. Municipal water and rainwater were commonly cited as ablution water sources (86%). Groundwater and lake water, were also considered acceptable (59%). In total, 18% (3/17) of samples from the patient's home, including one sample from the water heater, yielded *N. fowleri*; none of the three samples from the mosque yielded *N. fowleri*. Although the patient was likely exposed to *N. fowleri* at home, his primary water source mirrors those of other mosque members, demonstrating an ongoing risk for PAM in the USVI Muslim community. Culturally appropriate education materials on water treatment and PAM are needed.

884

IDENTIFICATION OF A CANDIDATE SEROTONERGIC RECEPTOR IN *ENTAMOEBA INVADENS*

Veena Ramachandran[1], Henry Pollack[1], Daniel Eichinger[2]

[1]Department of Pediatrics, New York University School of Medicine, New York, NY, United States, [2]Department of Microbiology, New York University School of Medicine, New York, NY, United States

Entamoeba histolytica, the etiologic agent of amebiasis, is one of the leading causes of parasitic disease, especially in resource limited countries and regions with poor sanitation. The life cycle of Entamoeba alternates between a feeding trophozoite and dormant cyst stage, the latter of which is passed from host to host, therein transmitting infection. The mechanism by which the parasite encysts, or transforms from the trophozoite to cyst form, is not well understood. Previous research has suggested the involvement of classical adrenergic, serotonergic, and histaminergic signaling pathways via G-protein coupled receptors (GPCR) and a cyclic AMP cascade. Agonists of these respective pathways were found to stimulate encystment while antagonists prevented encystment. The object of this study is to verify the presence of a potential serotonergic receptor and understand its role in the process of encystment when bound to serotonin-like agonists. Using the related species *E. invadens* as a model, the likely genomic sequence coding for the GPCR has been identified based on sequence alignment with known eukaryotic GPCRs. The gene has been PCR amplified and cloned into a pET102/D-TOPO® *E. coli* vector for bacterial expression and *in vitro* transcription/translation system expression. Expression of the protein in each system has been verified by western blotting. We will now use radiolabeled serotonin-like ligands to confirm the GPCR-ligand relationship, screen libraries of small molecules for novel antagonists, and determine the structure of the ligand binding site for future design of amoeba-specific inhibitors.

885

TOXOPLASMA GONDII: A REVIEW OF THE RELATIONSHIP BETWEEN SEROPOSITIVITY AND PSYCHIATRIC MORBIDITY

Carol D. Gerrin, David P. Adams

Armstrong Atlantic State University, Savannah, GA, United States

Toxoplasma gondii is an obligate protozoan parasite that has the ability to invade and infect all mammalian cells, except erythrocytes. Domestic cats are the definitive host, acquiring *T. gondii* by ingesting prey such as birds and rodents that are infected . The parasite may exist in three forms: oocyst, tachyzoite and bradyzoite. Human infection occurs from ingestion of contaminated food or water, unprotected exposure to litter boxes of acutely infected cats, and congenital transmission. *T. gondii* has been demonstrated to reside in neural and brain cells, based upon post mortem analyses that showed involvement of both hemispheres. Given the ability to invade and infect brain tissue, researchers have explored the possibility of a link between *T. gondii* infection and psychiatric disorders, particularly schizophrenia and major depression. The purpose of this review is to evaluate the current literature regarding a possible causal link between *T. gondii* infection and such psychiatric disorders.

886

DETECTION AND GENOTYPING OF *TOXOPLASMA GONDII* IN HIV AND BLOOD DONORS AT THE KORLE-BU TEACHING HOSPITAL, ACCRA

Roberta Koku[1], Senyo Kofi Fumador[2], Joshep Harold Osei Nyarko[3], Emmanuel Blay[4], Charles Brown[5], Anita Ghansah[3], Patrick Ayeh-Kumi[5], Irene Ayi[3]

[1]College of Agriculture and Consumer Sciences, University of Ghana, Legon, Accra, Ghana, [2]University of Ghana Medical School, Accra, Ghana, [3]Noguchi Memorial Institute for Medical Research, Legon, Accra, Ghana, [4]University of Ghana, Legon, Accra, Ghana, [5]School of Allied Health Sciences, Accra, Ghana

Detection and genotyping of *Toxoplasma gondii* in HIV infected individuals and blood donors at the Korle-Bu Teaching Hospital Accra Ghana. *T. gondii* is an obligate intracellular protozoan parasite which causes the disease toxoplasmosis. *T. gondii* infects through the gut, lung or broken skin. Cats (primary host) excrete oocysts, but the ingestion of poorly cooked infected meat by humans may also be as important as contact with cat feaces. In humans, the oocyst release trophozoites, which migrate widely with the prediction for eye, brain and muscle. Infection is live long and HIV may reactivate it. Purpose of study Detect and genotype *T. gondii* found in HIV infected individuals and blood donors visiting the Korle Bu Teaching Hospital Accra Methodology Blood samples (2-3ml) were collected from volunteers of HIV individuals and blood donors. DNA was extracted from the blood samples using the Qiagen blood and tissue kit. Nested (PCR) was performed using Sag 3 and Gra 6 primers. Samples that tested positive for Toxoplasmosis was further taken through restriction fragment length polymorphism using *Nci* I and *MSel* I restriction enzymes. To find the genotype that was present. Major findings Out of the 150 screened for HIV infected individuals 67/150 were positive for *Toxoplasma* representing 44.7%. Using Ncil and Msel restriction enzymes for genotyping 3/150, 2% positive for type I and 60/150, 40% for type II. The blood donors had 5/152, 3.3% positive for *T. gondii* and all were type II. In conclusion, the type II was common among the two groups, a few of types I was also found. The HIV infected individual who were found positive had their $CD4^+$ T cell count 0>200. A number of supposedly healthy blood donors were infected with *T. gondii,* but the blood donors donating at the blood bank are not been screened for *Toxoplasma gondii* and therefore there is a need to screen the blood donors before blood is been donated especially to the immunocompromised individuals.

887

PREVALENCE OF *TRICHOMONAS VAGINALIS* AMONG PREGNANT WOMEN IN KINSHASA, DEMOCRATIC REPUBLIC OF THE CONGO

Ange L. Landela

Kinshasa University, Kinshasa, Democratic Republic of the Congo

Trichomonas vaginalis is a flagellated protozoan causing sexually transmitted infection extremely common among the poor. It is cosmopolitan and is spread when hygiene measures are defective. It is also a cofactor in the transmission of HIV, a major cause of vaginitis in pregnant women and responsible for premature delivery, premature rupture of membranes, low birth weight children and infection of newborn. So we wanted to determine the prevalence of *T. vaginalis* among pregnant women. We conducted a cross-sectional study from November to December 2012 among pregnant women in Kinshasa. A sample of vaginal secretions swab was done, fixed on a slide and stained with Giemsa. Of 412 women enrolled, 96 patients were infected with *T. vaginalis* or 23.3%, whose average age was 28 ± 6 years. The infection was common among married women (55.6%), with a status of monogamous marriage (59.5%) whose spouse was trader (34.5%).

888

SEROPREVALENCE OF *TOXOPLASMA GONDII* IN WILD BOARS IN SOUTH KOREA

Wooseog Jeong

Animal and Plant Quarantine Agency (QIA), Anyang, Republic of Korea,

Toxoplasma gondii is an obligate intracellular protozoan parasite which is a significant human and veterinary pathogen. The wild boar (*Sus scrofa coreanus*) is regarded as a good indicator for monitoring environmental contamination with *T. gondii*. The purpose of this study was to survey the prevalence of antibodies against *T. gondii* in wild boars from South Korea. Blood samples were collected from 426 wild boars, which had been captured during 2008-2012 hunting seasons. Antibodies to *T. gondii* were detected in 152 samples of the 426 animals, indicating an overall seroprevalence of 35.7%. No correlation was found for any pairs between the prevalence of toxoplasmosis and the density of wild boar, or the rate of forest area (p>0.05 for all pairs).

889

ISOSPORA BELLI INFECTION WITH CHRONIC DIARRHEA IN AN ALCOHOLIC PATIENT

Jong-Yil Chai[1], Min Jae Kim[1], Woo Ho Kim[1], Hyun-Chae Jung[1], Jee-Won Chai[2]

[1]Seoul National University College of Medicine, Seoul, Republic of Korea, [2]Seoul Metropolitan Government-Seoul National University, Boramae Hospital, Seoul, Republic of Korea,

Chronic diarrhea with a 35 kg weight loss (75 kg to 40 kg) occurred during 2 years in an alcoholic patient was diagnosed with *Isospora belli* infection in the Republic of Korea. The patient, a 70-year old Korean male, had been a heavy drinker for more than 30 years. He was admitted to the Seoul National University Hospital because of long-standing diarrhea and severe weight loss. He had an increased white blood cell (WBC) count with high peripheral blood eosinophilia (36.8-39.9%) and lowered protein and albumin levels but without any evidence of immunosuppression. A parasitic infection was suspected and fecal examination was repeated 3 times with negative results. Peroral endoscopy with mural biopsy was performed in the upper jejunum. The biopsy specimens revealed villous atrophy with loss of villi together with various life cycle stages of *I. belli,* including trophozoites, schizonts, merozoites, macrogamonts, and microgamonts. The patient was treated successfully with oral doses of trimethoprim 160-320 mg and sulfamethoxazole 800-1,600 mg daily for 4 weeks. A follow-up evaluation at 2.5 years later revealed marked improvement of body weight (68 kg), increased protein and albumin levels, and normal WBC count with low eosinophils (3.1%). This is the first clinical case of isoporiasis with demonstration of various parasitic stages in the Republic of Korea.

890

ANTIPROTOZOAL ACTIVITY OF DEFENSINS

A. M. D. Zehrbach, Alexandra R. Rogers, D. Ellen K. Tarr

Midwestern University, Glendale, AZ, United States

Invertebrate defensins are part of the cysteine-stabilized alpha-beta superfamily that also includes defensins from plants and fungi, and some arthropod toxins. Defensins are usually considered to be most effective against bacteria, but some also show antifungal and/or anti-*Plasmodium* activity. Several arthropod species (*Aeshna cyanea, Drosophila melanogaster, Pandinus imperator*, and *Phrormia terranovae*), express defensins/defensin-like peptides with activity against malaria parasites. In addition, the tick *Haemaphysalis longicornis* expresses a defensin called longicin that inhibits transmission of *Babesia.* Both *Plasmodium* and *Babesia* are vector-borne blood parasites, suggesting these defensins might have evolved in the context of vector-pathogen interactions. However, a defensin-like peptide from *Anaeromyxobacter dehalogenans* also shows anti-*Plasmodium* activity, leading us to hypothesize that these peptides may have a more general antiprotozoal activity. A short sequence motif (five amino acids) in the m-loop has been hypothesized to be the basis of this activity. If this motif does confer antiprotozoal activity, we would expect to see it in defensins from a broad range of taxa and not limited to those with vector potential. Using information available from online, public sequence databases, we identified putative defensins with the hypothesized antiprotozoal motif in non-arthropod taxa, including nematode and tardigrade species. Studies with recombinant peptides are in progress to determine the spectrum of defensin antiprotozoal activity and verify that the hypothesized sequence motif is the biological basis for that activity.

891

ASSOCIATIONS OF ENTERIC PARASITE INFECTIONS WITH ROTAVIRUS-NEGATIVE DIARRHEA IN CHILDREN UNDER FIVE YEARS: A CASE CONTROL STUDY IN RURAL DISTRICT IN ECUADOR

Yosselin Vicuna[1], Stephanie Menzies[1], Carlos Sandoval[2], Nely Broncano[2], Fabian Salazar[2], Martha Chico[2], Philip Cooper[3]

[1]Laboratorio de Investigaciones FEPIS, Pontificia Universidad Catolica del Ecuador, Liverpool School of Tropical Medicine, Quininde and Quito, Ecuador, [2]Laboratorio de Investigaciones FEPIS, Pontificia Universidad Catolica del Ecuador, Liverpool School of Tropical Medicine, Quininde, Ecuador, [3]Laboratorio de Investigaciones FEPIS, Pontificia Universidad Catolica del Ecuador, Liverpool School of Tropical Medicine, Quininde, Quito, Ecuador, and Liverpool, United Kingdom

There are few data on the associations between enteric parasite infections and diarrhoea. To explore the associations between common enteric parasites and diarrhoea in pre-school children, we did a case control study of children aged 5 years or younger in a rural District of Ecuador. Children aged 6 months to 5 years were recruited at a pediatric outpatient clinic in the town of Quininde, Esmeraldas Province. Cases were children presenting with acute diarrhea (3 or more liquid stools in the previous 24 hours) that was negative for rotavirus infection by enzyme immunoassay (Prospect Rotavirus, Oxoid, UK), an assay that has bene calibrated to detect viral loads indicative of roatvirus diarrhea, and controls were healthy asymptomatic children being reviewed at the same clinic. A stool sample was collected from all children and was examined using the Kato-Katz and formol-ether concentration methods for *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm infections. An aliquot of stool was stored at -20C and analysed by real-time PCR for the presence of DNA for *Strongyloides stercoralis*, *Giardia lamblia*, *Entamoeba histolytica*, and *Cryptosporidium*. We evaluated 168 diarrhoea cases and 171 asymptomatic controls. The prevalence of infections among cases and controls were: *A. lumbricoides* (cases 11.1% vs. controls 9.3%); *T. trichiura* (9.3% vs. 9.3%); *S. stercoralis* (1.8% vs. 1.2%); *G. lamblia* (34.5% vs 28.1%), *Cryptosporidium* (4.08 vs. 0.6%, P=0.017). Only 1 child was infected with hookworm and none with *E. histolytica.* Although *Cryptosporidium* infection was detected in a small proportion of children aged under 5 years with mild to moderate diarrhoea, it was the only enteric parasite infection measured that was associated with diarrheal illness in our study population.

892

DETECTION OF ACUTE TOXOPLASMOSIS IN PREGNANT WOMEN BY INDIRECT HAEMAGGLUTINATION AND CONVENTIONAL NESTED PCR IN AL-MADINAH AL-MUNAWARAH, SAUDI ARABIA

Abdullah D. Al Hujaili[1], Mohamed A. Ramadan[2], Fawzia A. Habib[3], Howaida A. Al-Sabaa[4]

[1]College of Medical Rehabilitation Sciences, Nursing Department, Taibah University, Al-Madinah Al-Munawarah, Saudi Arabia, [2]College of Medical Rehabilitation Sciences, Nursing Department/Immuno-Molecular Parasitology Unit, Taibah University, Al-Madinah Al-Munawarah, Saudi Arabia, [3]College of Medicine, Obstetrics and Gynecology Department, Taibah University, Al-Madinah Al-Munawarah, Saudi Arabia, [4]College of Nursing, Maternity and Childhood Nursing Department, Taibah University, Al-Madinah Al-Munawarah, Saudi Arabia

Difficulties met during interpretation of serological tests carried out in pregnant women to detect primary maternal infection with toxoplasmosis during pregnancy implies more and more frequent use of the molecular technique, Polymerase Chain Reaction (PCR). To evaluate the degree of correlation between the results of a routine serological test and the *Toxoplasma gondii* genome in mother blood, conventional nested PCR a rapid, sensitive and specific molecular diagnostic technique was applied on 100 pregnant women from Al-Madinah Al-Munawarah, KSA, whose blood samples were analyzed for the presence of B1 gene of *T. gondii*. The presence of IgG and IgM *T. gondii* antibodies were analyzed by indirect haemagglutination (IHA) assay. The study revealed no agreement between the results of IHA and PCR. *T. gondii* genetic material in blood was found in 41 (41%) samples. IgG was detected in 10 cases of this PCR-positive samples. IgM was also detected in 6 of 10 samples. On the other hand, 59 (59%) cases were PCR-negative ; 32 of them were serologically positive and the remaining 27 cases were serologically negative. In a conclusion, the study clarifies the need of a confirmatory assay in addition to serology for detection of primary acute toxoplasmosis (misdiagnosed cases) in pregnant women. Nested PCR (semi quantitative) amplification of the B1 gene of *T. gondii* using whole blood is a rapid, sensitive and strain specific molecular diagnostic technique and cosiderable a value tool for establishing the diagnosis of *T. gondii* infection in adult females before or during pregnancy in Al-madinah al-Munawarah, KSA. Our future study to apply the Real Time PCR (RT-PCR) technique for fast detection of acute toxoplasmosis cases in Al-Madinah AL-Munawarah, KSA.

893

DEVELOPMENT OF REAL-TIME PCR FOR THE QUANTITATIVE AND SPECIFIC DETECTION OF *BABESIA DUNCANI* IN BLOOD

Robert E. Molestina, Melisa Birkan, Matthew Boley, Glaser Kathleen, Maria Mayda

ATCC - BEI Resources, Manassas, VA, United States

Babesia sp. are obligate protozoan parasites of red blood cells. Transmission to humans occurs through bites from infected ticks or blood transfusion. Infections with *B. microti*, which are endemic in the Northeast and upper Midwest regions of the United States, account for the majority of the reported cases of babesiosis in the US. The incidence of this disease is much lower in other US regions and is usually caused by the more recently described species *B. duncani*. The current gold standard for detection of *Babesia* is microscopic examination of blood smears. Recent PCR-based assays, including real-time PCR targeting the nuclear 18S ribosomal RNA gene, have been developed for *B. microti*. On the other hand, molecular assays that detect and distinguish the related *B. duncani* species are lacking. The greatest amount of sequence variation among related species of eukaryotes occurs within the internal transcribed spacer (ITS) regions of nuclear ribosomal RNA. Thus, in the present study, we targeted the ITS regions of *B. microti* and *B. duncani* to develop sensitive and species-specific real-time PCR assays. The assays were shown to discriminate *B. duncani* from *B. microti* and resulted in a limit of detection of 100 gene copies. Moreover, ITS real-time PCR for *B. duncani* was diagnostic in DNA extracted from blood of experimentally infected hamsters, detecting infections of low *parasitemia* that may potentially go undetected by microscopic examination (i.e., ≤0.01 % infected red blood cells). In summary, we have developed a sensitive and specific quantitative real-time PCR assay for the detection of *B. duncani* in blood. Our method could be used as a sensitive approach to monitor the progression of *parasitemia* in rodent models of infection as well as serve as a useful molecular test in blood screening.

894

DEVELOPMENT OF A QPCR-BASED METHOD TO QUANTIFY THE RATIO OF HOST-TO-PARASITE DNA IN *THEILERIA PARVA*-INFECTED HOST LYMPHOCYTES

Hanzel T. Gotia, **Joana C. Silva**

University of Maryland School of Medicine, Baltimore, MD, United States

Theileria parva is a tick-transmitted intracellular apicomplexan pathogen of cattle in sub-Saharan Africa. It causes East Coast fever (ECF), an acute fatal disease that kills over one million cattle annually, imposing a tremendous burden on many smallholder African farmers for whom cattle is the primary source of food and income. Conventional and quantitative real-time PCR (qPCR) are commonly used for the detection of parasite piroplasms in tissue biopsies but quantification is generally not pursued. One of the primary challenges to relative quantification is the miniscule amount of *T. parva* DNA in mixed DNA samples, in which host DNA prevails by >5 orders of magnitude. However, the ratio of host-to-parasite DNA in a sample is essential to determine the feasibility of obtaining high quality parasite genome sequence data through whole genome sequencing of samples of *T. parva*-infected host cells. We have developed an absolute quantification method based on qPCR where recombinant plasmid vectors containing genes specific for *T. parva* and *Bos taurus* are used as the quantification standards. The highly accurate detection of the number of target gene copies within the sample was achieved using two different primer sets, which were specifically designed to amplify the *T. parva* homolog of the *Plasmodium falciparum* apical membrane antigen 1 (AMA1) gene, as well as the bovine gene hypoxanthine phosphoribosyltransferase 1 (HPRT1). The qPCR values were then used to determine genome copy number and the genomic DNA equivalence ratio, given the relative size of the host and parasite genomes. Quantification of parasites combined with genomic equivalence ratio determination provides a simple and reliable method of assessing *T. parva* loads, which is essential for next-generation sequencing applications and may also play a vital role in studying host-parasite relationship and treatment efficiency.

895

SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTH INFECTIONS AMONG PRE-SCHOOL AGED CHILDREN IN MBITA, WESTERN KENYA

Maurice R. Odiere[1], Fredrick R. Ouma[1], William E. Secor[2], Diana M. Karanja[1], Pauline N. Mwinzi[1], Patrick J. Lammie[2], Kimberly Y. Won[2]

[1]Kenya Medical Research Institute, Neglected Tropical Diseases Branch, Kisumu, Kenya, [2]Centers for Disease Control and Prevention, Division of Parasitic Diseases and Malaria, Atlanta, GA, United States

Recent surveys have shown large numbers of preschool-aged children (PSAC) (≤5 years) infected with *Schistosoma mansoni*, yet PSAC are typically excluded from mass treatment campaigns. Our study determined the prevalence and intensity of *S. mansoni* and soil-transmitted helminth (STH) infections and association between infection and growth outcomes in children aged 1-5 years in Mbita, western Kenya. A total of 1,458 PSAC (712 boys, 746 girls) were examined. Single stool samples were examined by Kato-Katz for *S. mansoni* and STH eggs. Hematuria was used as a proxy indicator of *S. haematobium* infection and hemoglobin concentration was determined to assess anemia (< 10g/dL). Growth morbidities among

PSAC were determined from the WHO Child Growth Standards. Overall prevalence of *S. mansoni*, *S. haematobium* and STH was 28.2%, 31.4% and 3.9%, respectively. Of the 396 stool samples positive for *S. mansoni*, egg density was 258.9 ± 569.1; 241 (60.8%), 98 (24.8%) and 57 (14.4%) were light, moderate and heavy infections, respectively. The youngest infected child was one year of age. Stunted growth was observed in 21% of PSAC, of whom 7.4% were severely stunted. Underweight and wasting were observed in 8.8% and 3.1% of PSAC, respectively, and 22.7% of PSAC were malnourished. The prevalence of anemia, independent of infection (n = 1,165), was 30.2%; 67.1%, 29.6% and 3.4% had mild, moderate and severe anemia, respectively. Growth morbidities and anemia were not associated with *S. mansoni* infection. Our findings add to evidence that young children are at risk for *S. mansoni* infection, and that infections debut before school enrollment age in high endemic areas. With acquisition of infection early in life, children might not receive first treatment for up to 4 years after infection if present deworming policies are not revised. PSAC are a potential reservoir for transmission, emphasizing the need for their inclusion when designing control strategies.

896

PUROMYCIN ACTIVITY AGAINST *SCHISTOSOMA MANSONI* FOR DEVELOPMENT OF AN ANTIBIOTIC SELECTION SYSTEM MEDIATED BY RETROVIRAL TRANSGENESIS

Chuan Ju[1], Anne E. Folley[1], Danielle E. Skinner[1], Shuqi Wang[1], Victoria H. Mann[1], Zheng Feng[2], Wei Hu[3], Paul J. Brindley[1], **Gabriel Rinaldi**[1]

[1]George Washington University, Washington, DC, United States, [2]Key Laboratory of Parasitology and Vector Biology, National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, World Health Organization Collaborating Centre for Malaria, Schistosomiasis and Filariasis, Shanghai, China, [3]Department of Microbiology and Microbial Engineering, School of Life Science, Fudan University, Shanghai, China

Drug selection of transgenic schistosomes would be desirable as it would provide a means to enrich for populations of transgenic worms. We recently demonstrated that Murine Leukemia Virus (MLV) transduced schistosomes expressing neomycin phosphotransferase (NeoR) could be rescued using the aminoglycoside antibiotic, G418. In addition, after infecting snails with miracidia hatched from MLV-transduced eggs, sequencing of genomic DNA from cercariae released from the snails revealed the presence of transgenes, demonstrating that transgenes had been transmitted through the asexual developmental cycle, and confirming germline transgenesis. Moreover, the germline-transmitted transgenes encoding NeoR rescued cultured schistosomules from toxicity of the antibiotic G418. However, the aminonucleoside antibiotic puromycin has been shown to be faster and more efficient than G418 in selecting transgenic vertebrate cells (i.e. within 48 h). Accordingly, here we tested schistosome sensitivity to puromycin for eventual use in deriving selection of transgenic schistosomes via transduction of eggs with MLV carrying the puromycin resistance marker (PuroR). Schistosomules, eggs from liver or laid *in vitro* by adults, and sporocysts of *Schistosoma mansoni* were cultured in increasing concentrations of puromycin. Media and antibiotic were periodically replaced, and schistosomules and sporocysts were scored as live or dead by dual-fluorescence bioassay. Viability of schistosome eggs isolated from liver was evaluated by an egg hatch assay on days 5 and 10, whereas the development of the eggs laid *in vitro* was monitored microscopically every day and by egg hatch assay on day 7. Although eggs were insusceptible to G418, the developmental stages examined here were sensitive to puromycin. These findings will facilitate not only 'dual selection' of schistosomes with G418 and puromycin, but also the enrichment of MLV-transduced eggs and sporocysts that can be reintroduced in the life to cycle of the parasite augmenting the efficiency of the transgenic approach.

897

AN UNCEASING PROBLEM: PREVALENCE AND RISK FACTORS OF SCHISTOSOMIASIS AMONG CHILDREN IN YEMEN

Hesham M. Al-Mekhlafi, Hany Sady, Mohammed A. Mahdy, Nabil A. Nasr, Ahmed K. Al-Delaimy, Johari Surin

University of Malaya, Kuala Lumpur, Malaysia

Schistosomiasis, one of the most prevalent neglected tropical diseases, is a life-threatening public health problem worldwide. In Yemen, schistosomiasis is the second leading cause of death, after malaria, with an estimated 3 million people are infected. This study aims to determine the current prevalence and associated risk factors of schistosomiasis among children in rural Yemen. Urine and fecal samples were collected from 400 children. Urine samples were examined using filtration technique for the presence of *Schistosoma haematobium* eggs while fecal samples were examined using formalin-ether concentration and Kato Katz techniques for the presence of *S. mansoni* eggs. Demographic, socioeconomic, behavioral and environmental information were collected using a pre-tested questionnaire. Overall, 31.8% of the participants were found to be positive for schistosomiasis; 23.8% were infected with *S. haematobium* and 9.3% were infected with *S. mansoni*. The prevalence of schistosomiasis was significantly higher among children aged > 10 years compared to those aged ≤ 10 years ($P < 0.05$). Multivariate analysis confirmed that presence of other infected family member, low household monthly income, using unsafe sources for drinking water, living nearby stream/spring and living nearby pool/pond were the key factors significantly associated with schistosomiasis among these children. In conclusion, these findings support an urgent need to start an integrated, targeted and effective schistosomiasis control programme with a mission to move towards the elimination phase. Besides periodic drug distribution, health education and community mobilisation, provision of clean and safe drinking water, introduction of proper sanitation are imperative among these communities in order to curtail the transmission and morbidity caused by schistosomiasis.

898

CLINICAL AND ULTRASONOGRAPHIC CORRELATES OF HEPATOSPLENIC SCHISTSOMIASIS AMONG CHILDREN AND ADULTS IN A *SCHISTOSOMA MANSONI* HYPERENDEMIC RURAL AREA OF ZAMBIA

James Chipeta[1], Mable Mwale-Mutengo[2], Helen Chitundu[3], Takafira Mduluza[4], James Mwansa[2]

[1]University of Zambia School of Medicine, Department of Paediatrics and Child Health, Lusaka, Zambia, [2]University of Zambia School of Medicine, Department of Pathology and Microbiology, Lusaka, Zambia, [3]University of Zambia School of Medicine, Department of Radiology, Lusaka, Zambia, [4]University of Zimbabwe, Department of Biochemistry, Harare, Zimbabwe

The aim of schistosomiasis control programs is to reduce disease morbidity. However, comprehensive evaluation of these programs using available morbidity assessment tools is limited. Here we present clinical and ultrasonography correlates of hepatosplenic schistosomiasis among children and adults in a rural area of Zambia illustrating the limitations in these tools. Seven hundred fifty-four community members (159 children and 595 adults) from four rural locations (Mwadasengo, Luampa, Namando and Mangango) of Kaoma District had clinical asssesmments (September-October 2012) and were tested for *Schistosoma mansoni,* other helminthes, malaria, and haemoglobin status. Ultrasonography was done in 710(94%) participants to check for liver abnormalities. Of the 717 screened for parasitic infections, *Schistosoma mansoni*, hookworm, and malaria infection prevalence were 42%, 26.9%, and 6.5%, respectively. Twelve percent had hookworm-*S. mansoni* co-infection, 5.2% *S. mansoni*-malaria and 1.95% *S. mansoni*-hookworm-malaria multiple infections. On ultrasonography 72.4% had no periportal fibrosis (PPF),14.4% had mild while 7.2% and 6% had moderate and severe PPF, respectively. On logistic regression, sex-female (odds ratio [OR] = 2.0; 95% CI= 1.26,

3.34), age ≥44 (OR = 5.86; 95% CI = 1.70, 20.25), area-Namando (OR = 4.73; 95% CI = 1.62, 13.86), and PPF-(p <0.001) were associated risk factors for splenomegaly while, intensity of infection and other parasitic infestations were not. On the other hand, only age group 34-44 (OR = 3.25; 95% CI = 1.50, 7.05) and age group ≥44 (OR = 3.20; 95% CI =1.43, 7.14) were strong associated risk factors for PPF. Of note was the strong correlation between PPF severity with ultrasonographic portal vein diameter (r=0.56, p-value <0.001) and weakly so for hepatomegaly(r=0.29 p <0.001).Collectively, these findings affirm the limitations of the available schistosomiasis morbidity assessment tools. There is urgent need to devise standardized clinical criteria or indeed search for molecular markers for the accurate assessment of schistosomiasis morbidity.

899

DETERMINATION OF TEMPERATURE EFFECTS ON SUSCEPTIBILITY OF *BULINUS TRUNCATUS TRUNCATUS* SNAILS TO *SCHISTOSOMA HAEMATOBIUM* AND ASSOCIATED MORTALITY AND FECUNDITY

Elizabeth Perez[1], Matthew Tucker[2]

[1]George Washington University School of Public Health and Health Services, Washington, DC, United States, [2]Biomedical Research Institute, Rockville, MD, United States

One obstacle in laboratory maintenance of *Schistosoma haematobium* is inherent resistance among the intermediate host *Bulinus spp.*, where infection rates in the laboratory are as low as 30%. A previous study in the *B. glabrata/S. mansoni* system showed heat exposure of snails prior to parasite infection can alter susceptibility. We studied effects of increased temperature on the susceptibility of *B. t. truncatus* to *S. haematobium* by exposing them to heat (32ºC) prior to infection. Three distinct snail size groups were used: neonates (≤1-2mm length), pre-adults (≥2-3mm), and adults (>8mm). Over 10 weeks post-infection, we monitored infection rates and effects on fecundity of *B. t. truncatus*. Across size groups, an inverse relationship between size and susceptibility was observed. Comparison of ambient temperature vs. heat-treated snails in each size group found heat-exposure increased infection rates ~2-fold in neonate and pre-adult snails. Adult snails were relatively non-susceptible. In the neonate and pre-adult groups, increased susceptibility due to heat-exposure correlated with lower mortality compared to non-heat treated snails. During patency, the mean weekly fecundity ratio (#egg sacs/surviving patent snails) of heat-treated neonates and ambient temperature pre-adults was significantly less than matched uninfected snails (p<0.05). No significant difference was observed in the mean weekly fecundity ratio between patent ambient vs. heat-treated neonates and pre-adults. Our results suggest that temperature manipulation can alter susceptibility of young *B. t. truncatus* snails to *S. haematobium* and it may have a protective effect for survival. Heat-exposure affected neonates the most for increased susceptibility but also decreased fecundity in these snails. Although pre-adult snails exhibited less susceptibility than neonates, they tolerated heat stress better in terms of fecundity and mortality. These results have implications for optimization and improving propagation of the *S. haematobium* life cycle, which will be valuable for future schistosomiasis research.

900

HEPATOSPLENIC *SCHISTOSOMIASIS MANSONI* DISEASE BURDEN IN ZAMBIA: CLINICAL AND LABORATORY EVALUATION TO DETERMINE PHENOTYPES FOR CYTOKINE GENE-POLYMORPHISM ANALYSIS

Mable Mwale-Mutengo[1], Takafira Mduluza[2], Helen Chitundu[3], James Mwansa[1], James Chipeta[4]

[1]University of Zambia School of Medicine, Department of Pathology and Microbiology, Lusaka, Zambia, [2]University of Zimbabwe, Department of Biochemistry, Harare, Zimbabwe, [3]University of Zambia School of Medicine, Department of Radiology, Lusaka, Zambia, [4]University of Zambia School of Medicine, Lusaka, Zambia

Globally, schistosomiasis is a chronic parasitic disease of major public health importance being endemic in 78 countries with at least 243 million people reported to have required treatment in 2011.Chronic *Schistosoma mansoni* infection can present into a wide spectrum of clinical syndromes with various degrees of severity. Several studies have delineated the aetiopathogenesis of chronic schistosomiasis disease syndromes to be due to schistosomal elicitation of cross-regulatory innate, Th1, Th2, and Th17 immune responses in the host. Immune responses attributable to *Schistosoma mansoni* infections are reported to be under host genetic control. Elucidating the immuno-genetic background to these immune responses, in the human host, will not only provide further understanding of schistosomiasis immunopathology but may herald novel effective ways of combating the disease. Here we describe the design, clinical and laboratory evaluations of a cross-sectional study cohort of individuals in a *schistosoma mansoni* hyperendemic rural Zambia set to segregate phenotypes for hepatosplenic schistosomiasis cytokine gene-polymorphism analysis.

901

EFFECT OF HISTONE DEACETYLASE INHIBITOR ON GENE EXPRESSION PROFILES AND HISTONE ACETYLATION IN *SCHISTOSOMA MANSONI*

Leticia Anderson[1], Marina de Moraes Mourão[2], Guilherme Oliveira[2], Raymond Pierce[3], Sergio Verjovski-Almeida[1]

[1]Universidade de São Paulo, São Paulo, Brazil, [2]Centro de Pesquisa René Rachou, Belo Horizonte, Brazil, [3]Institut Pasteur de Lille, Lille, France

Histone modifying enzymes such as histone deacetylases (HDACs) have a key role in the epigenetic regulation of gene expression, and their potential as new therapeutic targets was evidenced with the apoptotic phenotype that resulted from *S. mansoni* HDAC inhibition by Trichostatin A (TSA). In order to characterize gene expression changes caused by *S. mansoni* treatment with TSA, microarray experiments were performed. Total RNA was extracted from schistosomula (LE strain) treated for 12, 24, 48 and 72 h either with 1µM TSA or with ethanol (vehicle) as a control. Triplicate samples were labeled with either Cy3 or Cy5 and hybridized to custom-designed Agilent oligoarrays. Gene expression analyses showed 870 (12h), 968 (24h), 703 (48h) and 188 (72h) genes with statistically significant differential expression (q-value<0.05) and fold-change >2. Ingenuity Pathway Analysis (IPA) showed gene networks significantly enriched with differentially expressed genes related to DNA replication, cell cycle, cell death and survival, protein synthesis and nucleic acid metabolism. At 12 and 24 h treatment, genes related to DNA replication, recombination and repair were up regulated. Interestingly, genes related to and regulated by Polycomb repressive complex suggest that this complex has a decreased activity after 12 h treatment of schistosomula, which could affect the chromatin methylation status. Moreover, western blotting with total protein lysates from adult worms treated with TSA showed hiperacetylation at H2A-Lys5, H2B-Lys12, H3-Lys9 and H4-Lys5. This study provides important data for understanding the response of the parasite to hiperacetylation of histones and regulation of gene transcription.

902

DIFFERENCES IN THE EXPRESSION OF HSP70 STRESS PROTEIN BETWEEN JUVENILE *BIOMPHALARIA GLABRATA* SNAILS THAT ARE EITHER SUSCEPTIBLE OR RESISTANT TO *SCHISTOSOMA MANSONI*

Oumsalama Elhelu[1], Wannaporn Ittiprasert[2], Andre Miller[2], Nithya Raghavan[1], Carolyn Cousin[1], Matty Knight[3]

[1]University of the District of Columbia, Washington, DC, United States, [2]Biomedical Research Institute, Rockville, MD, United States, [3]George Washington University, Washington, DC, United States

The stress protein Heat shock protein 70 (Hsp 70) is constitutively expressed in the *Schistosoma mansoni* intermediate snail host, *Biomphalaria glabrata*. Susceptible stocks of *B. glabrata* allow miracidia to freely develop after penetrating the soft tissue parts of the snail host. Thus, after shedding its cilia plates the miracidium developme, United Statesnt in the susceptible NMRI snail, progresses from mother to daughter sporocyst stages, permitting asexual reproduction of the free- swimming cercarial stage. In the parasite- resistant juvenile snail, such as BS90, however, invading parasites are encapsulated and killed not long after penetration. Mechanism(s) shaping these outcomes involves the parasites ability to evade the snail's innate defenses. By Western blot analysis using anti-snail recombinant Hsp 70 antiserum, soluble protein extracts from NMRI and BS-90 snails exposed to *S. mansoni* for different time points was examined. Results showed early and strong induction of Hsp70 protein in susceptible but not resistant snails. These data will aid in understanding how a parasite -mediated stress response, involving transcriptional regulation of Hsp 70, and juvenile snail susceptibility helps with the development of intra-molluscan stages of *S. mansoni*.

903

THE FEASIBILITY STUDY OF THE *ONCOMELANIA* SNAIL DETERMINATION OF SCHISTOSOMIASIS BY GPS AND GOOGLE EARTH

Wenlong Pi, Xiaodong Tan, Zengwang Zheng, Yuzhong Liu, Ziling Ni

Wuhan University, Wuhan, China

In endemic areas of schistosomiasis, the oncomelania snail survey is one the key measure to control schistosomiasis. The current *Oncomelania* snail survey lacks accuracy and repeatability of the snail spots, and manual workload is very heavy after the survey. To explore to reduce the manual workload and improve the accuracy by applying GPS and Google earth. This study used 3 methods to solve the problems: 1 Compare the feasibility on different positioning techniques such as GPS, maps, softwares in *Oncomelania* snail survey. 2 Synthesis of Positioning technology. 3 Feasibility study and case analysis: Systematic verification on Yangtze River bund. Finally, the study attained the following findings: 1 Use the Google earth to ascertain the environment in advance and decide the sampling techniques of snail survey. 2 Use the handheld GPS to locate the work track and the snail spot in particular and gather the surrounding photographs. 3 Import/export in batch the data of GPS(not input/export one by one), innovate the data expression and get the environment database and geographic information map. 4 The current location accuracy is about 50 meters, and this study limit the accuracy within 3 meters. The current data process usually needs 5 days, but this study just needs half a day. To conclude, this study makes the task of snail survey clearer, the location more accurate, the snail spot more repeatable, the data process more efficient, the snail distribution more direct, the data sharing more convenient, and lay a reliable basis for monitoring and early warning of snail status.

904

RISK ASSESSMENT OF ACUTE SCHISTOSOMIASIS OUTBREAK AROUND THE RENOVATION OF YANGTZE RIVER BUND BY DECISION TREE METHOD

Zengwang Zheng, Xiaodong Tan, Wenlong Pi, Yuzhong Liu, Ziling Ni

Wuhan University, Wuhan, China

It is generally believed that it is not suitable for the breeding of snails after the hardening and renovation of Yangtze River bund. With effective control of schistosomiasis in the past years, the vigilance of residents living on the banks of the Yangtze River is decreasing year by year. But snail reproduction (no infectious) was founded, The objective of the study is to make a scientific evaluation design by the decision tree method, the observational method and the bowknot method. To achieve the above objectives, the authors analyze characteristics of mainstream people on the Yangtze River bund, determined the existing risk factors, classified relevant risk factors by observational, decision tree and bowknot methods respectively. Finally, the authors got the following risk assessment study design: Firstly, social behavioral observational method was used to investigate the spatial pattern of mainstream people on the Yangtze River bund. Secondly, systematic sampling and GPS was combined to accurately position infectious snail distribution on the Yangtze River bund. Thirdly, the Delphi method and risk matrix method was applied to assess the occurrence possibility and harmfulness of acute schistosomiasis infection. After conducting a trial by using the above methods on the Yangtze River bund,this scheme proved feasible, and the main target groups were identified,the snail distribution was clear,and the results risk evaluation is reliable.

905

CHARACTERIZATION OF THE BAS-CONGO VIRUS GLYCOPROTEIN AND ITS FUNCTION AND USE IN PSEUDOTYPED VIRUSES

Imke Steffen[1], Nathan M. Liss[1], Bradley S. Schneider[2], Joseph N. Fair[2], Charles Y. Chiu[3], Graham Simmons[1]

[1]Blood Systems Research Institute, San Francisco, CA, United States, [2]Metabiota, San Francisco, CA, United States, [3]University of California San Francisco, San Francisco, CA, United States

The novel rhabdovirus Bas-Congo virus was first identified from a survivor of a small hemorrhagic fever outbreak in the Bas-Congo province of the Democratic Republic of Congo (DRC). However, it was not possible to culture the virus and a reverse genetics assay is still under development. Using only the BASV glycoprotein (BASV-G) we established a pseudotype system based on a glycoprotein-deficient vesicular stomatitis virus (VSV) vector to study BASV-G driven membrane fusion and viral entry into target cells without replication-competent virus. In vitro studies revealed that BASV-G displayed a broad tissue and species tropism similar to its well-studied relative VSV-G. While BASV-G mediated membrane fusion was pH-dependent, acidification in the absence of a target membrane did not lead to inactivation of the viral fusion protein. This suggests that the conformational changes induced in BASV-G by low pH are fully reversible. This data and structural features identified by comparative sequence similarity analyses with other rhabdovirus glycoproteins support the classification of BASV-G as a class III viral fusion protein. Notably, transition of BASV-G into the fusion active conformation required a lower pH and higher temperatures than those observed for VSV-G. Another distinction from VSV-G was the finding that BASV-G contained high-mannose glycans that allowed binding to certain C-type lectins, thereby enhancing its attachment to target cells. The BASV-G pseudotype assay described in this study will be an important tool in the absence of an infectious cell culture assay for BASV. It will facilitate future investigations of BASV-G mediated cell entry and its inhibition, as well as serology testing required to uncover the prevalence and importance of BASV as a potential novel human pathogen in the DRC and throughout Central Africa.

906

BIOSAFETY AND BIOSECURITY CHALLENGES IN CONTROLLING VIRAL HEMORRHAGIC DISEASES OUTBREAKS IN UGANDA

Julius J. Lutwama

Uganda Virus Research Institute, Entebbe, Uganda

Uganda lies in a region where many viral diseases, leading to a lot of debilitation and deaths, have emerged in the recent past. Lying at the equator and being at the confluence of the Equatorial Forest zone of West Africa and the Tropical Savannah zone of Eastern Africa it hasa good climate, many freshwater rivers and lakes, and a diverse flora and fauna favourable for many insect vectors and reservoirs of viral infections. Particularly alarming are the VHFs: Ebola Virus fever, Marburg Virus fever, Yellow fever, Dengue fever and others that have been recorded in Uganda in the recent past. A lot of training in biosafety has been emphasized over the years but biosecurity is a new term and often confused with biosafety. Several programs to promote biosafety and biosecurity have been introduced in the country. However, there are many challenges to implementation of these programs which are developed in the western world. There are limited resources and infrastructure to support and implement them in a systematic and sustainable manner. Simply getting core concepts and procedures into the hands of those working with biological agents and processes is a significant challenge. Many of the facilities handling infectious agents in developing countries were built more than 30 years ago, with little or limited provision for biosafety and biosecurity in terms of both design and practice. The conditions found in the majority of these facilities remain shocking to those operating in laboratories in developed countries. Shortcomings in capacity, equipment and human resources pose a weak link in the chain of control against the misuse and abuse of infectious agents to inflict harm. Other challenges include: short comings in bioethics and accountability, poor remuneration of personnel, loss of research materials, loss of collaboration opportunities, loss of funding opportunities, loss of training opportunities, loss of property rights and loss of control of our activities and facilities. There is need to increase governments efforts to improve the quality of facilities. We have been training personnel nationally and internationally; we have improved our biosafety and biosecurity in institutions handling highly infectious pathogens. We are developing a national policy on biosafety and biosecurity and we have also formed a biosafety and biosecurity association to address some of the challenges.

907

FATAL NEUROLOGICAL DISEASE IN RATS AND NON-HUMAN PRIMATES AFTER INHALATIONAL EXPOSURE TO RIFT VALLEY FEVER VIRUS

Amy L. Hartman[1], Diana S. Powell[1], Laura M. Bethel[1], Amy L. Caroline[1], Jacquelyn M. Bales[1], Timothy D. Oury[2], Douglas S. Reed[1]

[1]University of Pittsburgh Regional Biocontainment Laboratory, Center for Vaccine Research, Pittsburgh, PA, United States, [2]University of Pittsburgh School of Medicine, Department of Pathology, Pittsburgh, PA, United States

Rift Valley Fever virus (RVFV) outbreaks occur in Africa and the Middle East, resulting in severe disease in livestock and human populations. In humans, RVFV causes a self-limiting febrile illness, but some people develop severe complications including encephalitis. RVFV is infectious by many routes, including inhalation. Animal models that mimic human disease can be used as tools to understand the pathogenesis and test the efficacy of potential countermeasures. Up to this point, there have been no well-characterized rodent or NHP models for the encephalitic form of RVF. In the current studies, ACI inbred rats, African Green Monkeys (AGM), and marmosets were exposed to small particle aerosols containing RVFV, and all developed fatal neurological disease. ACI rats develop a fever and encephalitis after either s.c. inoculation or inhalation of RVFV, although 1,000 - 10,000-fold more virus is required to cause lethality by s.c. infection compared to aerosol. ACI rats have little peripheral virus replication but high levels of virus are found in the brain. Granulocytic leukocytosis was seen in the blood during the course of infection. Using radiotelemetry, both NHP species demonstrated a marked fever response after infection. Five out of six (5/6) AGM and four of eight (4/8) marmosets developed neurological signs (drooling, unsteady gait, seizures) and became moribund. The median lethal dose in marmosets was estimated to be 3,500 pfu. This is the first documented evidence of reproducible, severe disease seen in NHPs after infection with RVFV. High levels of infectious virus were found in brain and spinal cord, and both species displayed elevated white blood counts, which consisted primarily of granulocytes. Pathological findings confirmed viral encephalitis in both AGMs and marmosets. In conclusion, ACI rats, AGM, and marmosets all develop neurological disease after aerosol exposure to RVFV that is similar to that seen in humans. All can serve as appropriate tools to understand the pathogenesis and evaluate therapeutics against inhalational Rift Valley Fever.

908

SEROEPIDEMIOLOGICAL STUDIES AND ASSESSMENT OF PREVELANCE OF TICK-BORNE ENCEPHALITIS AND CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS IN KAZAKHSTAN

P.N. Deryabin[1], T.I. Nurmahnov[1], A.N. Vilkova[1], J. Hay[2], R. Hewson[3], B.B. Atshabar[1], V.I. Sapozhnikov[1], A.M. Matzhanova[1], S.B. Isayeva[1], A.I. Belyaev[1], B. Moldabekov[1], Z.T. Mamanova[1], A.C. Aymukhametova[1]

[1]Kazakh Scientific Center for Quarantine and Zoonotic Diseases, Almaty, Kazakhstan, [2]University of Buffalo, Buffalo, NY, United States, [3]Health Protection Agency, United Kingdom

In Kazakhstan, the Almaty province is endemic for tick-borne encephalitis (TBE), but has no human cases of Crimean-Congo hemorrhagic fever (CCHF) registered. The Kyzylorda province is endemic for CCHF, but has no human cases of TBE registered. The objectives of the study were to assess CCHF and TBE antiviral antibodies in individuals residing in endemic and non-endemic areas, and to assess CCHF and TBE infection rates among ticks collected from those same areas. We tested 500 serum samples from healthy individuals residing in four regions of Almaty province and five regions of Kyzylorda province for the presence of IgG antibodies using ELISA. We also examined 5463 ticks (264 pools) in the genera *Hyalomma*, *Haemaphysalis* and *Dermacentor*, collected in the same regions, for the presence of viral antigen using ELISA and viral RNA using RT-PCR. In the Almaty province, IgG antibodies to TBE virus were found in 18% of all serum samples. There were no samples positive for CCHF antiviral antibodies. CCHF-infected ticks were found in one region and TBE infected ticks were found in two regions. In the Kyzylorda province, IgG antibodies to TBE virus were found in 2.4% of all serum samples. 7.3% of all serum samples in this province were positive for CCHF, while 1.7% samples were positive for both TBE and CCHF. Prevalence of TBE virus infection among ticks collected in all regions of Kyzylorda province varied from 0% to 1.15%, while CCHF prevalence was between 0% and 2.16%. Thus, antibodies to TBE virus were found in individuals residing both in endemic Almaty province and non-endemic Kyzylorda province. CCHF antiviral antibodies were detected only among samples from Kyzylorda. The highest infection rate of TBE and CCHF in ticks and the highest level of presence of TBE and CCHF antibodies in humans coincided in the same regions. Clearly, the extent of human exposure to TBE virus has been underestimated in Kazakhstan.

909

DEVELOPMENT OF A MOSQUITO TRAP THAT USES SUGAR FEEDING TO DETECT EASTERN EQUINE ENCEPHALITIS VIRUS

Andrea M. Bingham, Nathan D. Burkett-Cadena, Thomas R. Unnasch

University of South Florida, Tampa, FL, United States

Eastern equine encephalomyelitis virus (EEEV) is the most pathogenic arbovirus endemic to the USA. Prevention of infection relies upon transmission surveillance and community-wide prevention measures to prevent the spread of the virus to humans. Many counties in Florida cannot afford the costs associated with thorough active surveillance, including testing of wild birds, sentinel chickens, and mosquito pools. For mosquito surveillance, sample size is extremely important due to low infection rates in mosquito populations. Current methods rely on mosquito pools with no greater than 50 mosquitoes and can be costly and time consuming. We designed a surveillance system that exploits virus secretion in saliva during sugar feeding by mosquitoes. Modified collection chambers of CO_2-baited traps are supplied with honey-coated nucleic acid preservation cards. Mosquitoes that feed upon honey expectorate viral particles onto the card which are then inactivated and preserved by the card. RNA extracted from the cards can then be screened via RT-PCR for arboviruses. This method will allow us to screen more mosquitoes at a time, decreasing the amount of labor and cost. In field trials, we found that 1) the modified traps captured as many females with a similar species distribution as did standard CO2-baited CDC light traps; 2) nearly all females (91.4%) in traps fed on honey; and 3) traps could run unattended for 3 consecutive days on a single battery and CO_2 tank. Experimental inoculations of EEEV onto honey coated preservation cards demonstrated that viral levels down to 1 PFU were detectable for up to seven days. Additional field trials are currently in progress and will be included in the data presented at the conference.

910

A FILOVIRUS MARATHON: EPIDEMIOLOGICAL AND LABORATORY RESPONSES TO TWO OUTBREAKS OF MARBURG AND EBOLA HEMORRHAGIC FEVER, UGANDA, OCTOBER-NOVEMBER 2012

Barbara Knust[1], Ilana Schafer[1], Trevor Shoemaker[2], Nyakarahuka Luke[3], Joseph Wamala[4], Esther Muwanguzi[4], Charles Okot[5], Zabulon Yoti[6], Armand Bejtullahu[7], Jonathan Polonsky[8], Estrella Lasry[9], Hilde DeClerck[10], Ardith Gibbons[1], Kimberly Dodd[11], Shelley Campbell[1], Cesar Albarino[1], Stephen Balinandi[2], Alex Tumusiime[2], Ruth Aceng[4], Ute Stroeher[1], Stuart T. Nichol[1], Pierre E. Rollin[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Centers for Disease Control and Prevention, Entebbe, Uganda, [3]Uganda Virus Research Institute, Entebbe, Uganda, [4]Uganda Ministry of Health, Kampala, Uganda, [5]World Health Organization, Kampala, Uganda, [6]World Health Organization, Brazzaville, Republic of the Congo, [7]World Health Organization, Geneva, Switzerland, [8]EpiCentre, Paris, France, [9]Medicins Sans Frontieres, New York, NY, United States, [10]Medicins Sans Frontieres, Brussels, Belgium, [11]University of California Davis, Davis, CA, United States

On October 18, 2012, blood specimens from three patients in the Kabale District of Uganda were laboratory-confirmed for Marburg virus infection. National and international agencies responded by rapidly establishing active case finding, contact tracing and follow-up, a field diagnostic laboratory, an isolation ward, and outreach to the affected communities. Marburg Hemorrhagic Fever (MHF) case-patients were defined as confirmed cases with laboratory detection of Marburg virus or antibodies, while probable cases had clinical illness and epidemiologic linkages in the absence of laboratory testing. A total of 26 confirmed and probable MHF cases in 3 districts were identified, with the first illness onset dating to July 2012--3 months prior to the outbreak's detection. Case fatality rate was 58%. There were two distinct chains of transmission, both occurring in Ibanda district at the same time. Sequence analysis of patient specimens from both chains found 99.9% matching in sequence identity of virus strains, indicating a single outbreak. In the midst of the MHF response, 2 patients with Sudan virus infection were detected in the Luwero district of Uganda on November 13. Response efforts were quickly diverted to this outbreak. A total of 7 Ebola Hemorrhagic Fever (EHF) case-patients were identified, 6 of which were laboratory-confirmed, and 4 who died (Case fatality rate = 57%). In contrast to the MHF outbreak, the first identified EHF case-patient had illness onset approximately 1 month prior to the outbreak's detection. In both outbreaks, timely mobilization of resources occurred, and all case-patients were put into isolation within 2 weeks. The origin of the epidemic--presumed to be zoonotic transmission of filovirus from infected animals to humans--was investigated but not identified in either outbreak. Early identification and halting of direct human-to-human transmission contributed to the successful containment of these filovirus epidemics; the presence of diagnostic capacity in country, coupled with clinician awareness was crucial to prevention of additional cases and deaths.

911

BURDEN AND SEVERITY OF INFLUENZA INFECTIONS IN YOUNG ANDEAN CHILDREN

Philip J. Budge[1], Marie R. Griffin[1], Kathryn M. Edwards[1], John V. Williams[1], Hector Verastegui[2], Stella M. Hartiger[3], Ana I. Gil[2], Claudio F. Lanata[2], Carlos G. Grijalva[1]

[1]Vanderbilt University, Nashville, TN, United States, [2]Instituto de Investigacion Nutricional, Lima, Peru, [3]Swiss Tropical and Public Health Institute, Basel, Switzerland

We conducted a prospective household-based study of acute respiratory illness (ARI) among children in the rural Peruvian Andes. We used these data to characterize the burden of influenza-associated ARI (IA-ARI), identify risk factors for influenza infections, and compare the clinical characteristics of IA-ARI with other ARI etiologies. We followed a dynamic cohort of 892 children age <3 years in the highland villages of San Marcos Province, Cajamarca, Peru, from May, 2009 to September, 2011. Trained field workers visited the home of each child weekly, collected data on respiratory symptoms, and assessed symptomatic children for danger signs including: wheezing, retractions, grunting, nasal flaring, stridor and tachypnea. Nasal swabs collected from children with ARI (cough or fever) were analyzed for respiratory viruses by monoplex real-time RT-PCR. The incidence of IA-ARI by influenza types (A, B and C) was calculated by Poisson estimation. Characteristics of IA-ARI and non-IA-ARI were compared using two-proportion z or Wilcoxon rank-sum tests. Influenza vaccination status for each child was determined. The overall incidence of IA-ARI was 34/100 child-years. Influenza A incidence was highest (20/100 child-years), followed by influenza B (9/100) then influenza C (5/100). Influenza circulated seasonally, with moderate peaks in July - October, 2009 and June - December, 2010, and a small peak in August-September, 2011. Pandemic H1N1 accounted for 46% of influenza A: 89% (33/37) in 2009, 27% (30/111) in 2010, and 100% (7/7) in 2011. When compared with non-IA-ARI, IA-ARI were longer in duration (median 6.5 vs. 5 days, $p < 0.001$), with more days of fever (median 2 vs. 1, $p<0.001$), cough (5 vs. 4 days, $p<0.001$), and malaise (median 2 vs. 0 days, $p < 0.001$). IA-ARI were not more frequently associated with danger signs (6.9% of IA-ARI vs. 7.5% of others, $p=0.77$) or hospitalization (2.5% vs. 2.7%, $p=0.91$), but IA-ARI more frequently resulted in health care center visits (38% vs. 24%, $p < 0.001$). Influenza vaccination coverage for the observed seasons was too low to assess vaccine effectiveness. Influenza burden is high among Andean children and associated with longer illness duration and higher health care utilization than non-influenza ARI. Influenza C circulated in the study settings but its burden was low relative to other influenza types. Improvements in influenza vaccine delivery in this population are needed.

912

SYMPTOMATIC AND ASYMPTOMATIC NOROVIRUS INFECTIONS DURING THE FIRST THREE YEARS OF LIFE IN A TROPICAL ECUADORIAN BIRTH COHORT

Tarak K. Trivedi[1], Benjamin Lopman[2], Yosselin Vicuna[3], Andrea Alevalo[3], Carlos Sandoval[4], Martha Chico[4], Nely Broncano[4], Maritza Vaca[4], Fabian Salazar[4], Nicole Gregoricus[2], Jan Vinje[2], Philip Cooper[5]

[1]Fundación Ecuatoriana Para Investigación en Salud, Centers for Disease Control and Prevention, Quininde, Ecuador, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Fundación Ecuatoriana Para Investigación en Salud, Pontificia Universidad Catolica del Ecuador, Quininde and Quito, Ecuador, [4]Fundación Ecuatoriana Para Investigación en Salud, Quininde, Ecuador, [5]Fundación Ecuatoriana Para Investigación en Salud, Pontificia Universidad Catolica del Ecuador, Liverpool School of Tropical Medicine, Quininde, Ecuador

Noroviruses are recognized as a major cause of acute gastroenteritis worldwide. In some developed countries using rotavirus vaccination, noroviruses are the most common cause of pediatric hospitalization for gastroenteritis. With upcoming norovirus vaccines, it is important to understand the incidence of endemic, community-based disease and the development of natural immunity to norovirus infection and diarrhea. We monitored 194 Ecuadorian infants born from March-December 2009 from birth until 3 years by weekly phone calls, clinic surveillance, and stool collections during diarrhea episodes and diarrhea-free periods at scheduled home visits. Noroviruses were quantified by genogroup-specific real-time, quantitative PCR. 1360 samples were collected from 194 children over 3 years of life, with a mean of 8.68 samples per child. 432 (32%) samples were collected during episodes of acute diarrhea. Norovirus was detected in 248 (18%) of all samples, but was not found more frequently in diarrhea samples (79/432; 18.2%) than routine samples (169/ 928; 18.3%, p=0.973). However, viral loads (indicated by cycle threshold (Ct) values) were higher in diarrhea samples (median Ct = 27.9) compared to routine samples (Ct = 32.2; Wilcoxon rank-sum p = 0.004). The incidence of all cause diarrhea was 94.7/100 child-years (95% CI: 85.2 - 105.1) while that of norovirus-positive diarrhea was 21.5/100 child-years (95% CI: 17.2 - 26.8). 39% of norovirus strains were genogroup 1 and 61% were genogroup 2 (n = 248). Norovirus infection prevalence was strongly associated with age (p<0.001): 2% (4/176) at 0-5 months; 24% (185/764) at 6- 23 months; and 14% (59/420) 24-35 months. 3% (4/128) of children experienced a primary infection by 6 months, 35% (49/139) by 1 year, 62% (87/140) by 2 years, and 74% (104/140) by 3 years. Norovirus infections were strongly associated with age, and a majority of children had at least one infection by age 3. Although norovirus prevalence was not associated with disease, viral load was, with higher levels in diarrhea cases in this tropical community-based birth cohort.

913

TRANSCRIPTOME SEQUENCING REVEALS ELEVATED IMMUNE GENE EXPRESSION IN DEER MICE INFECTED WITH ANDES VIRUS

Tony Schountz[1], Andrew Caudill[2], Travis Glenn[2], Timothy I. Shaw[2], Elaine Haddock[3], Heinz Feldmann[3], Joseph Prescott[3]

[1]Colorado State University, Fort Collins, CO, United States, [2]University of Georgia, Athens, GA, United States, [3]Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, United States

Hantavirus cardiopulmonary syndrome (HCPS) is a cytokine-mediated disease with a high case-fatality rate that is caused by several New World hantaviruses. Each virus is naturally hosted by a principal rodent species without conspicuous disease, and once infected the rodents remain infected, perhaps for life. Deer mice (*Peromyscus maniculatus*) are the natural reservoir hosts of Sin Nombre virus (SNV), an etiologic agent of HCPS. Despite a helper T cell response that leads to high titered neutralizing antibodies, deer mice remain persistently infected. Deer mice are also susceptible to Andes hantavirus, which causes HCPS in South America; however, deer mice clear ANDV. We infected 5 deer mice with SNV and 5 deer mice with ANDV and examined lymph node cell antigen recall responses by deep transcriptome sequencing using the Illumina MiSeq and HiSeq platforms. We obtained about 1 million reads per sample from the MiSeq, and obtained a mean of 44 million reads per sample from the HiSeq with a range of 33 million to 54 million reads. We assembled the transcriptomes using Trinity. Analysis of MiSeq data from the two groups identified 80 genes that were significantly different between cultures from SNV and ANDV infections. All but two of the transcripts were more abundant in cultures from ANDV-infected deer mice than those from SNV-infected deer mice. Most of the transcripts have immune functions, while some are not known to have immune function. About half of the transcripts were related to interferon and/or antiviral responses, indicating that such responses occur with greater magnitude in deer mice infected with ANDV than SNV. We are continuing to analyze the HiSeq data to identify additional differentially expressed transcripts, identify differences between individual deer mice and to map the genes to functional pathways. Together with our previous work, these data suggest the magnitude of the immune response is substantially greater during ANDV infection compared to SNV infection, and may account for clearance of ANDV.

914

THE CONTRIBUTION OF HERD IMMUNITY TO THE EPIDEMIC CYCLES OF RIFT VALLEY FEVER VIRUS IN SOUTH AFRICA

Melinda K. Rostal, William B. Karesh, Parviez Hosseini

EcoHealth Alliance, New York, NY, United States

Rift Valley fever (RVF) causes devastating outbreaks in both humans and domestic animals, leading to significant morbidity, mortality and economic instability. While epidemics of RVF virus (RVFV) and correlate closely with climate and NDVI (normalized difference vegetation index) in East Africa, the climate models are less effective predicting outbreaks in southern or western Africa. We hypothesize that the herd immunity of ruminants (both wild and domestic) can affect the severity, duration and location of RVFV outbreaks. We developed a dynamic mosquito-host-infection model for integration within the current climate models to better predict the occurrence and spread of RVF epidemics in South Africa. Our model includes *Aedes* sp mosquitoes, which transmit RVFV transovarially, *Culex* sp mosquitoes, which spread RVFV from infected ruminants to susceptible ruminants or humans, and ruminants, including parameters such as age, previous exposure and vaccination. When forced with a combination of ENSO-like periodicity (4-7 year) for mosquito abundance and annual seasonal variation of the mosquito population, these equations can produce periodic epizootics of RVF in the ruminant population. This demonstrates that herd immunity may have an important role in the occurrence of an RVF outbreak, which likely changes depending on management regime (culling and vaccination) and the most recent RVF outbreak. This model, in conjunction with the climate models, could improve the prediction of outbreaks in regions with varying climate, unlike that of East Africa. This model could also potentially be used to predict the movements and likelihood of outbreaks under climate change scenarios.

915

RISK FACTOR ANALYSIS FOR NIPAH INFECTION IN BANGLADESH, 2004 TO 2012

Sonia T. Hegde[1], Hossain M. Sazzad[2], M. Jahangir Hossain[3], Eben Kenah[4], Peter Daszak[5], Mahmudur Rahman[6], Stephen P. Luby[7], Emily S. Gurley[2]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [3]Medical Research Council Unit, The Gambia, Gambia, [4]University of Florida, Gainsville, FL, United States, [5]EcoHealth Alliance, New York, NY, United States, [6]Institute of Epidemiology Disease Control and Research, Bangladesh, Dhaka, Bangladesh, [7]Stanford University, Palo Alto, CA, United States

Since 2001, human Nipah encephalitis outbreaks have been identified almost yearly in Bangladesh. Over 70% of infected persons die. Raw or fermented date palm sap consumption and person-to-person transmission have been identified as major transmission pathways. However, many identified case-patients have had neither of these exposures based on epidemiologic surveys, suggesting alternative pathways of transmission that have not been identified in individual outbreak investigations due to limited statistical power related to small sample sizes. We conducted a risk factor analysis using data from all investigated 157 cases from 2004-2012 and their geographically-matched controls to maximize our power to identify rarer exposures associated with being a Nipah case. Using conditional fixed effects logistic regression, we first generated univariate models, adjusting for age, sex, date palm sap consumption and contact with a sick case. We then built multivariate models based on these results and selected the best fitting model using the Akaike information criterion; associations were considered significant at $p<0.05$. Median age of case-patients was 26 (range 3 months to 95 years) years, 48% were female, and 22% reported no exposure to date palm sap or contact with a Nipah case-patient. In the multivariate model, case-patients were 3.3 (95% CI:1.2 - 8.8) times more likely to live near trees where Pteropus bats visit at night and 3.5 (95% CI: 1.5 - 8.22) times more likely to travel outside their sub-district than controls; we found no significant associations with eating dropped fruits or contact with animals. Most Nipah case-patients die; finding knowledgeable proxy respondents to ascertain exposures of Nipah case-patients who travelled is difficult. The most likely explanation for the association with travelling is that exposures to date palm sap or other Nipah cases likely occurred during travel but went unreported. The association with bats around the house at night is likely another indicator of bat contamination of date palm sap. While pathways of transmission other than date palm sap consumption and person-to-person transmission may exist, our evidence suggests that these are not major contributors to human Nipah infection in Bangladesh. These findings underscore the importance of focusing Nipah prevention efforts on interventions to interrupt transmission through date palm sap consumption and person-to-person contact.

916

ANTIBODIES TO POLIO IN GAMBIAN SUBJECTS: DO OUR VACCINATION PROGRAMS PROTECT ENOUGH?

Olubukola T. Idoko, Elishia Roberts, Momodou Cox, Jainaba Njie-Jobe, Martin Ota, Beate Kampmann

Medical Research Council, Banjul, Gambia

Vaccination remains one of the most cost effective interventions in preventing serious infectious diseases, including polio. As we move toward polio eradication additional oral polio vaccine (OPV) doses have been included in the form of boosters and supplemental immunization campaigns, primarily given to children under the age of 5. It is important to assess the impact of such vaccine programmes not only in young children but also in older children and adults in order to minimise susceptibility. The concentration of polio antibodies in children above 5 years who received several OPV doses and that of young adults vaccinated several years earlier with fewer OPV doses has not been previously assessed in our setting. Such information is crucial to assess the impact any polio outbreak would have in The Gambia and in evaluating the need for alternative vaccination schedules to facilitate polio eradication. This study was undertaken determine antibody persistence in 5 to 6 year olds compared with adults in The Gambia. Samples from 223 healthy 5 to 6 year olds and 83 individuals 18years or above were tested for polio antibodies using an established seroneutralization assay against polio 1 and 3. Titres ≥ 1:8 were considered protective. Preliminary results show 95% and 85% of fully vaccinated 5 to 6 year old had protective antibody concentrations to Polio serotypes 1 and 3 respectively. Fewer adults had protective antibody concentrations to Polio 1 (85%) with less protection against Polio 3 (80%). In conclusion, data suggest that multiple OPV doses given to young children are producing the desired effect with some herd effect in adults. Some adults may however remain vulnerable.

917

EVIDENCE OF CIRCULATION OF SELECTED ARBOVIRUSES IN IJARA AND MARIGAT DISTRICTS, KENYA

Victor O. Ofula[1], Joseph Oundo[2], Zephaniah Irura[3], Edith Chepkorir[4], Caroline Tigoi[4], Olivia Lwande[4], Juliette Ongus[5], Randal Schoepp[6], Cindy Rossi[6], Eyako K. Wurapa[1], Hesbon Sonde[7], Rosemary Sang[8]

[1]United States Army Medical Research Unit-Kenya, Nairobi, Kenya, [2]United States Army Medical Research Unit-Kenya, Kericho, Kenya, [3]Ministry of Health, Nairobi, Kenya, [4]International Centre for Insect Physiology and Ecology, Nairobi, Kenya, [5]Jomo Kenyatta University of Agriculture and Technology, Juja, Kenya, [6]United States Army Medical Research Institute for Infectious Diseases, Fort Detrick, MD, United States, [7]Ministry of Health, Ijara, Kenya, [8]Kenya Medical Research Institute, Nairobi, Kenya

Arboviruses are transmitted by arthropods with humans becoming infected during blood feeding by infected mosquitoes, ticks and sandflies. Characterization of arbovirus circulation and transmission in industrialized countries has been well documented, but there are many knowledge gaps in developing nations. Entomological surveys conducted so far have indicated circulation of arboviruses of significant public health importance in *Aedes*, *Anopheles* and *Culex* species in vast populations in Kenya, suggesting the presence of competent vector systems. The human involvement in the transmission cycle of these viruses has however not been demonstrated. This study sought to determine the sero-prevalence of a range of arboviruses including; Chikungunya, Dengue, Sindbis, Sandfly Naples, Sandfly Sicilian, Uganda S, West Nile and Zika viruses in Ijara and Marigat Districts where vector surveillance has been done. A total of 351 patient serum samples were analyzed using IgG ELISA, of these 193 (54.9%) were male and 158 (45.1%) were female with age ranging between 3 and 73. The overall arbovirus prevalence was 53/351 (15.1%) with a prevalence of 7% (10/143) in Marigat and 21% (43/208) in Ijara. Of the positives, Flaviviruses were 69%, alpha viruses 29.6% and Bunyaviridae 1.4%. Uganda S virus was the most prevalent with 10%, followed by West Nile virus 6%, Sindbis 5%, Dengue 2%, Chikungunya 1.1%, Sandfly Naples 0.2% respectively. Semliki-forest virus-specific antibodies were detected by plaque reduction neutralization test in 3/351 (0.85%) persons tested. Antibodies against Sandfly Sicilian and Zika viruses were not detected. This study constitutes the first detection of antibodies against Sandfly Naples virus in Kenya. The study has demonstrated the circulation of the selected arboviruses in the two sites amongst human population. These findings will improve our understanding of impact of Arboviruses on public health in the regions so that preventive actions and awareness among clinicians in patient' recognition and management can be enhanced.

918

ASSESSING HEPATITIS AND VACCINES IN A RESOURCE-LIMITED SETTING: THE PERSPECTIVES IN PREGNANCY AND MATERNAL DEATH

Chinedu O. Oraka

College of Health Sciences, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria

Acute viral hepatitis globally now seems to be a major cause of illness and of death in the developing world and disproportionate cause of deaths among pregnant women. Although, there are accounts of hepatitis vaccine trials that have shown candidate vaccines to be effective and well-tolerated, they are yet to be made available in sufficient quantities to susceptible populations. Verbal interviews and epidemiologic evidence were adopted on the pregnant women visiting the clinics of 2 rural maternity homes/centres in Isseke and Ihiala (both in Anambra State, South East Nigeria). Community survey showed exposure to hepatitis A virus during childhood. Hepatitis C is rare. Hepatitis B is endemic to the rural community, but the incidence of infection and illness are low. Hepatitis E remains the most likely cause of the pregnant women hepatitis-like illnesses. In this context, 17 deaths in women of reproductive age (during pregnancy or within 42 days postpartum) were recorded during July 2012 - February 2013 from both maternal homes. Verbal autopsies identified hepatitis, jaundice and hepatic failure as the primary cause of death in 12 (70.6%) of the 17 pregnancy-related deaths. In conclusion, some data suggests about 10% of deaths observed in pregnancy and in women of reproductive age could be linked to hepatitis. It is then pertinent to demonstrate the effectiveness and safety of the vaccines in the rural settings. To avoid a substantial portion of preventable deaths in these resource-limited settings, judicious and timely implementation of these vaccine interventions would be beneficial.

919

CASE REPORTS AND SEARCH-BASED SURVEILLANCE REVEAL PREDICTABLE, STRONGLY IMMUNIZING DYNAMICS OF HAND, FOOT AND MOUTH DISEASE (HFMD) IN SOUTHEAST ASIA

Tiffany L. Bogich[1], Sebastien Ballesteros[2], H. Rogier van Doorn[3], Bryan Grenfell[2], Jeremy Farrar[3]

[1]National Institutes of Health RAPIDD Fellow/Princeton University, Princeton, NJ, United States, [2]Princeton University, Princeton, NJ, United States, [3]Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam

Hand Foot and Mouth Disease (HFMD), caused by enteroviruses (primarily EV71, CA16) is a major and increasing cause of serious disease in Southeast Asia. Recently, there has been an alarming increase in the number of patients and an increase in the number of cases complicated by central nervous system and cardiopulmonary involvement and deaths in young children in countries across Southeast Asia in particular. However, the violent epidemic dynamics of these pathogens, and hence the prospects for control with vaccination, are ill understood. We address this problem with a synthesis of public health and web search surveillance time series using a novel platform for epidemiological inference. First, we use lab-confirmed cases from Japan to demonstrate considerable predictability (1-4 years) for EV71 and CA16 epidemics; the associated results indicate strongly immunizing dynamics for both pathogens. Lab-confirmed reports are more sparse for other countries. We address this with Google Trends searches, calibrated first against the Japanese surveillance data; the Google data successfully capture the dynamics of the disease, as the sum of EV71 and CA16. This allows us to extend the analysis more broadly, dissecting the signal of the two pathogens in other countries in the region. Results indicate a relatively optimistic scenario for vaccination, since these infection dynamics reflect sterilizing immunity and relatively moderate transmissibility. More broadly, this synthesis of traditional and novel surveillance with accessible inference provides a powerful tool for clarifying the dynamics of emerging infections.

920

CO-CIRCULATION OF *ORTHOBUNYAVIRUSES* INCLUDING NGARI VIRUS AMONG VECTORS IN ARID AND SEMI-ARID ZONES, KENYA

Rosemary Sang[1], Caroline Ochieng[2], Albert Nyunja[2], Joel Lutomiah[2], Albina Makio[2], Hellen Koka[2], Edith Chepkorir[3], James Mutisya[2], Edith Koskei[2], Olivia Wesula Lwande[3], Collins Odhiambo[3], Silvanos Mukunzi[2], Joshua Bast[4], Eyako Wurapa[2]

[1]Kenya Medical Research Institute, Nairobi, Kenya, [2]United States Army Medical Research Unit-Kenya, Nairobi, Kenya, [3]International Centre of Insect Physiology and Ecology, Nairobi, Kenya, [4]United States Army Medical Research Unit-Kenya, Kisumu, Kenya

Orthobunyaviruses are mosquito-borne viruses that have been associated with febrile illness and hemorrhagic fever in Kenya and elsewhere in Africa. Ngari virus was linked to 27% of the hemorrhagic fever cases during an outbreak of Rift Valley Fever (RVF) in Kenya and Somalia in 1997/98. We have continued to conduct vector-based surveillance to monitor Arbovirus circulation in general and looking out for co-circulation of *Orthobunyaviruses* that would lead to the reassortment and subsequent emergence of viruses like Ngari Hemorrhagic Virus (NRIV). Mosquitoes and ticks sampled between 2009-2010 in Kenya have been screened for Arboviruses by cell culture isolation and identified by RT-PCR. Eleven *Orthobunyavirus* isolates were further analyzed by RT-PCR and sequencing. Isolates of Bunyamwera, Pongola and Illesha were detected and interestingly, four isolates from ticks; *Amblyomma gemma, Rhipicephalus pulchellus* and mosquitoes; *Anopheles gambiae*, *Aedes macintoshi* were identified as Ngari virus sampled from Isiolo, Garissa and Tana-Delta. Other *orthobunyaviruses* were isolated from *Anopheles funestus, Mansonia african* and *Mansonia uniformis* from Magadi and Baringo. It is also noteworthy that we are yet to detect Batai virus, whose M segment constitutes Ngari virus. It is evident that important *Orthobunyaviruses,* including Ngari virus, an emerging hemorrhagic fever virus in E. Africa are circulating widely in parts of Kenya with potential for spread and possibly reassortment through co-infections and movement of animals with ticks. These viruses should be included as differentials in HF diagnosis in the region.

921

RAPID POINT-OF-CARE DETECTION OF SEVEN HEMORRHAGIC FEVER VIRUSES USING REVERSE TRANSCRIPTION RECOMBINASE POLYMERASE AMPLIFICATION ASSAY - TIME TO REACT FAST TO OUTBREAKS

Ahmed Abd El Wahed[1], Doris Heidenreich[1], Pranav Patel[2], Oumar Faye[3], Matthias Niedrig[2], Amadou A. Sall[3], Frank T. Hufert[1], Manfred Weidmann[1]

[1]University Medical Center, Goettingen, Germany, [2]CBS1-Highly Pathogenic Viruses, Center for Biological Threats and Special Pathogens, Robert Koch Institute, Berlin, Germany, [3]Arbovirus Unit, Pasteur Institute, Dakar, Senegal

The most widespread method used for the detection of nucleic acids of etiological agents in clinical samples is real time polymerase chain reaction. During outbreak, clinical samples are sent long distances to the laboratory for analysis, hence real time cyclers are not suitable for point-of-care diagnostics because it is heavy, big, expensive, complex, and must be managed by qualified staff. In addition, test run time is between 60-90 minutes. This study describes the development of real time reverse transcription recombinase polymerase amplification (RT-RPA) assays for the detection of Rift Valley fever virus, Ebola virus, Sudan virus, Bundibugiyo virus, Marburg virus, Dengue Virus, and Yellow fever virus. Quantitative RNA molecular standards were generated and used to determine RT-

RPA assays sensitivity. The analytical sensitivities of RT-RPA assays ranged from 16 to 3778 RNA molecules detected (probit analysis). RT-RPA was performed at 42°C and yielded results after 2 to 15 minutes. The assays showed neither cross-detection of any of the target genomes of the panel nor of the human genome. To test the possibility of using RT-RPA assays for outbreak investigations, a mobile RT-RPA unit was operated in Kedougou, in Senegal. A magnetic-bead based total nucleic acid extraction method was combined with RT-RPA on a portable instrument (tubescanner). Dried pellet RT-RPA reagents and dried oligonucleotides were used. The whole RT-RPA panel was successfully used to detect viral RNA extracted from gamma-irradiation inactivated lyophilised virus supernatants. All operations were carried out at an ambient temperature of 38°C with auxiliary electricity tapped from a vehicle. In conclusion, the developed assay protocol and mobile setup performed well and should be useful for rapid highly sensitive and specific detection of the haemorrhagic fever viruses in outbreak investigations.

922

HIGH RIFT VALLEY FEVER SERO-PREVALENCE IN PEOPLE DURING INTER-EPIDEMIC PERIOD IN THE KILOMBERO VALLEY, TANZANIA

Robert D. Sumaye[1], Dirk Berkvens[2], Mbaraka Amuri[3], Etienne Thiry[4], Eveline Geubbels[1]

[1]Ifakara Health Institute, Ifakara, United Republic of Tanzania, [2]Institute of Tropical Medicine, Antwerp, Belgium, [3]Jhpiego, Dar es Salaam, United Republic of Tanzania, [4]University of Liege, Liege, Belgium

Rift Valley fever (RVF) occurs in outbreaks in cycles of 5-15 years in the Eastern Africa region and the Horn of Africa, following unusual high rainfall that leads to sustained flooding. In recent years, evidence of RVF transmission during inter-epidemic periods (IEP) in Africa has been reported. The IEP transmissions generally pass undetected in absence of surveillance in mammalian hosts. We studied presence of and the determinants for IEP RVF transmission among humans in an area experiencing annual flooding in southern Tanzania. A sero-survey was conducted among members (n=606) of randomly selected households in 6 villages where a previous study in livestock indicated hotspots of RVF transmission, approximately 5 years after the 2006/07 RVF outbreak in Tanzania. The exposure status to RVF virus (RVFV) was determined using commercial ELISA kits, detecting IgM and IgG antibodies in serum. Information on risk factors for exposure was obtained through structured interviews with the participants. The risk factors were examined by fitting a logistic regression model with villages as random effects where a p-value ≤0.05 was considered statistically significant. An overall seroprevalence of 11.7% (95% CI 9.2-14.5) was recorded with increased exposure in older individuals (OR 1.03; 95% CI 1.01-1.04), in people who milked animals (OR 2.19; 95% CI 1.23-3.91), and individuals eating raw meat (OR 4.17; 95% CI 1.18-14.66). Keeping livestock was not associated with individuals' seropositivity but households keeping livestock had high chances of having at least one member exposed (OR 2.8; 95% CI 1.27-6.17). RVFV IgM antibodies were detected in 4.4% (95% CI 2.9-6.4). Sex, slaughtering, eating meat from dead animal, drinking raw milk, help animal birthing, net use and disposal of aborted fetus were not associated with seropositivity. These preliminary findings indicate IEP transmission with age and interaction with livestock being important risk factors. The high exposure in older individuals and demonstration of IgM antibodies implies a continuous exposure of the population.

923

THE INTERACTOME OF SCHIZONT EGRESS ANTIGEN-1, A NOVEL VACCINE CANDIDATE FOR *FALCIPARUM* MALARIA

Christina E. Nixon[1], Rute C. Silva[2], Dipak K. Raj[3], Christian P. Nixon[4], Christen DiPetrello[5], Jeffrey Dvorin[6], Jonathan D. Kurtis[3]

[1]Brown University/The Miriam Hospital, Providence, RI, United States, [2]Brown University, Providence, RI, United States, [3]Brown University/Rhode Island Hospital, Providence, RI, United States, [4]Yale University, New Haven, CT, United States, [5]Harvard University, Boston, MA, United States, [6]Harvard University/Boston Children's Hospital, Boston, MA, United States

Schizont egress is a complex and tightly regulated process that requires coordination of calcium-signaling, phosphorylation, and proteolysis leading to processing of both parasite and host RBC proteins. Central events include activation of PfPKG, release of PfSUB1 into the parasitophorous vacuole, proteolytic processing/activation of PfSERA5 by PfSUB1, and phosphorylation of unknown substrates by PfCDPK5. We discovered PfSEA-1 using a differential screening approach with plasma from children who were resistant or susceptible to *falciparum* malaria. Antibodies to the immunorelevant region of PfSEA-1 (rPfSEA-1A, aa 810-1083) predict resistance to severe disease in two yr old children, block schizont egress from infected RBC *in vitro,* and vaccination with rPbSEA-1A protects mice from *Plasmodium berghei* ANKA challenge. Moreover, PfSEA-1 localizes to the inner RBC leaflet consistent with a role in remodeling the RBC cytoskeleton prior to rupture. We have explored the protein-protein interactions of PfSEA-1 by screening a yeast two-hybrid Pf3D7 library with rPfSEA-1A as bait followed by confirmation with immuno-precipitation/mass spectroscopy. We have identified 15 interacting proteins including 5 putative substrates and 3 confirmed substrates (PfSERA5, PfRAP-1, and PfRhopH3) of PfSUB1, a protease critical for egress. In *in vitro* phosphorylation assays, we have demonstrated that rPfCDPK5 phosphorylates rPfSEA-1A. Together, these data support the role of PfSEA-1 in the complex schizont egress signaling pathway and suggest additional targets to augment the vaccine efficacy of rPfSEA-1A.

924

MULTIPLE PATHWAYS OF MOSQUITO MIDGUT INVASION BY *PLASMODIUM* OOKINETES

Joel Vega-Rodriguez[1], Anil K. Ghosh[2], Stefan M. Kanzok[3], Rhoel R. Dinglasan[1], Sibao Wang[4], Nicholas Bongio[5], Marcelo Jacobs-Lorena[1]

[1]Johns Hopkins University, Baltimore, MD, United States, [2]Case Western Reserve University, Cleveland, OH, United States, [3]Loyola University, Chicago, IL, United States, [4]Chinese Academy of Sciences, Shanghai, China, [5]Duquesne University, Pittsburgh, PA, United States

Plasmodium ookinete invasion of the mosquito midgut is a crucial step of the parasite life cycle. However, the molecular basis for this process is poorly understood. Previous studies from our laboratory showed that the Salivary gland and Midgut peptide 1 (SM1) binds to the lumenal surface of the mosquito midgut epithelium and that this binding results in inhibition of ookinete midgut invasion. Moreover, we found that SM1 is a mimotope of *Plasmodium* surface enolase. Recently, we have shown that both SM1 and ookinete surface enolase bind to a protein on the lumenal surface of the mosquito midgut termed Enolase-Binding Protein or EBP. Based on these and other observations, we hypothesize that ookinete midgut invasion involves the interaction of ookinete enolase and EBP. Cloning of *P. berghei* ANKA 2.34 parasites led to isolation of clones sensitive to SM1 inhibition and clones resistant to SM1. Midgut invasion by ookinetes of SM1-sensitive parasites, but not of the resistant, was blocked by anti-*Anopheles gambiae EBP* (AgEBP) antibodies or by AgEBP gene knockdown. In a separate set of experiments we determined that a peptide from the original phage display screen, Midgut Peptide 2 (MP2), blocks midgut invasion by both SM1-resistant and SM1-sensitive parasites. Moreover, unlike SM1, MP2 also efficiently inhibited midgut invasion by *P. falciparum* ookinetes, suggesting that MP2 binds to a "universal receptor"

required for midgut invasion by ookinetes from most or all *Plasmodium* species. Together these results provide the first evidence that *Plasmodium* ookinetes invade the mosquito midgut epithelium by more than one pathway, as is the case for blood stage merozoites. Characterization of the pathways of *Plasmodium* midgut invasion may lead to new transmission-blocking strategies.

925

CATCHING *PLASMODIUM FALCIPARUM* BETWEEN A ROCK AND A HARD PLACE: SUPPRESSING ANTI-MALARIAL DRUG RESISTANCE WITH AN EVOLUTIONARY TRAP

Leila S. Ross[1], Amanda K. Lukens[2], Francisco Javier Gamo Benito[3], Maria Jose Lafuente Monasterio[3], Michael Booker[4], Dyann F. Wirth[1], Roger C. Wiegand[2]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Broad Institute, Cambridge, MA, United States, [3]GlaxoSmithKline, Tres Cantos, Spain, [4]Genzyme Corporation, Waltham, MA, United States

Drug resistance emerges in an ecological context where fitness costs restrict the diversity of escape pathways. These pathways are targets for drug discovery. Blocking the most fit resistance pathways by combining wild-type and mutant-type inhibitors may prevent the emergence of competitively viable resistance. We tested this hypothesis with malaria parasites derived by *in vitro* selection with dihydroorotate dehydrogenase (PfDHODH) inhibitors. We selected *Plasmodium falciparum* lines resistant to structurally unrelated PfDHODH inhibitors (Genz-666136 and DSM74), and found a point mutation in PfDHODH (E182D). We discovered a compound more potent against E182D than wild-type parasites (IDI-6273). Mutant-specific drugs allow us to set a evolutionary trap: if resistance to one compound greatly increases sensitivity to another, then parasites that become resistant to one compound will be battered by the second. Selection of the E182D mutant with IDI-6273 gave a second nucleotide mutation in pfdhodh. This mutation gave a reversion to the wild-type protein sequence and drug-sensitivities. Combination selection with a wild-type PfDHODH inhibitor and IDI-6273, the mutant-selective inhibitor, did not yield resistant parasites in eighty days - ample time for every position in the genome to be mutated at least once. Recombinant wild-type and E182D PfDHODH protein mirrored the drug sensitivities seen in whole-cell assays, and also demonstrated that the E182D mutant has little effect on substrate affinity. This may explain why E182D is a favored mutation pathway, as other nearby mutations have larger detrimental effects on enzyme activity and thus overall fitness. We believe that evolutionary fitness constraints allow few pathways to resistance, and these pathways can be anticipated and preemptively blocked. The combination of well-chosen anti-malarial agents active against sensitive and resistant parasites effectively kills parasites in the short-term, and in the long-term, can help shape parasite evolution away from the development of drug resistance.

926

A NEW CLASS OF ANTIMALARIAL COMPOUNDS TARGETING THE NON-MEVALONATE PATHWAY ENZYME ISPD

Christopher Armstrong[1], Neil Berry[2], Paul O'Neill[2], **Audrey R. Odom**[1]

[1]Washington University School of Medicine, St. Louis, MO, United States, [2]University of Liverpool, Liverpool, United Kingdom

Antimicrobial drug resistance is an urgent problem in control and treatment of many of the world's most serious infections, including *Plasmodium falciparum* malaria and tuberculosis. Because the non-mevalonate pathway of isoprenoid biosynthesis is essential in eubacteria, mycobacteria, and *P. falciparum*, but not present in humans, there is great interest in targeting the enzymes of this pathway for antimicrobial and antiparasitic drug development. Our previous studies indicated that the enzyme IspD (E.C. 2.7.7.60; methylerythritol cytidyltransferase) was a point of particular metabolic control of isoprenoid biosynthesis in *P. falciparum* malaria parasites. We aimed to develop new lead compounds for antimalarial drug development targeting Pf-IspD. Our strategy relies on chemoinformatic modeling and in silico compound selection prior to physical screening of compounds against purified recombinant enzyme. We optimized high-throughput, reproducible assay conditions for Pf-IspD. We screened 5,000 diverse compounds, which were selected on the basis of predicted activity and promising ADMET properties from the large compound library available at Biofocus. From the most active of our initial hits, a series of compounds have been developed that inhibit the growth of cultured *P. falciparum* (IC_{50} of approximately 1 μM) with encouraging structure activity relationships. Metabolic analysis of compound-treated malaria cultures demonstrates inhibition of isoprenoid biosynthesis, confirming mechanism of action. Initial cellular toxicity studies demonstrate limited toxicity to human cell lines. Ongoing studies include medicinal chemistry efforts to improve compound activity and rescreening of additional Biofocus compounds to identify further chemical series. Our studies demonstrate the feasibility of targeting Pf-IspD for antimalarial development and have identified a new, promising class of antimalarial compounds.

927

GENOMIC SCALE EXPERIMENTAL GENETICS TOOLS FOR *PLASMODIUM BERGHEI*

Julian C. Rayner, Ellen S. Bushell, Frank Schwach, Burcu Anar, Gareth Girling, Oliver Billker

Wellcome Trust Sanger Institute, Cambridge, United Kingdom

The high AT-content of *Plasmodium berghei* genomic DNA precludes the production of large and complex genetic modification vectors by conventional restriction/ligation cloning into high-copy, circular plasmids. Two recent developments have converged to help us overcome this problem. Firstly, using a low copy, linear plasmid we generated the first high integrity genomic DNA library (PbG), which covers >90% of *P. berghei* genes on large genomic inserts of 9 kb on average. An arrayed library of end-sequenced PbG clones is now searchable online and clones are available as a free resource. Secondly, we developed a molecular tool kit and a set of protocols to modify PbG inserts using the Red/ET recombinase system from lambda phage, as reported previously. I will illustrate how recombinase-mediated engineering ("recombineering") of PbG clones can be used by individual laboratories to generate different genetic modification constructs, ranging from simple knock out and tagging vectors to more complex conditional alleles that use the FLP/frt system, as well as allelic exchange and complementation vectors for large genes that could not have been made using conventional techniques. Recombineered vectors have a much improved recombination frequency thanks to their long homology arms and all methods used for their production are sufficiently robust to scale to a 96-well format. Recognising these unique advantages, the Malaria Programme at the Wellcome Trust Sanger Institute has initiated the PlasmoGEM project, which aims to produce and distribute genome-wide, free resources of *Plasmodium* genetic modification vectors (http://plasmogem.sanger.ac.uk). We initially aim to generate and share the largest possible set of vectors for the targeted deletion and c-terminal epitope tagging of *P. berghei* genes. Vectors will be ready for marker recycling by negative selection and will feature gene-specific barcodes to serve as signature tags in genetic screens. The current status of the resource will be presented, along with its applications to genetic screens that could encompass the entire *Plasmodium berghei* genome.

928

PLASMODIUM GAMETOCYTES DENSITIES IN BURKINA FASO, IMPLICATIONS FOR IMPLEMENTATION OF TRANSMISSION-BLOCKING INTERVENTIONS

Dari F. Da[1], Melissa C. Kapulu[2], Serge R. Yerbanga[1], Ibrahim Sangaé[1], Bienvenue K. Yaméogo[1], Thomas S. Churcher[3], Andrew M. Blagborough[3], Jean Bosco Ouédraogo[1], Robert E. Sinden[3], Adrian V. Hill[2], Sumi Biswas[2], Anna Cohuet[4]

[1]Institut de Recherche en Sciences de la Santé (IRSS/DRO), Bobo-Dioulasso, Burkina Faso, [2]Jenner Institute, Oxford, United Kingdom, [3]Imprerial College, London, United Kingdom, [4]Institut de Recherche pour le Dévéloppement, Montpellier, France

Plasmodium gametocytes, the sexual-stage parasite in human blood are the transmissible forms to the *Anopheles* mosquito vectors, and thus mediate the onward transmission of the malaria. Breaking the human to mosquito transmission is a new promising concept of malaria control. To date, some transmission-blocking vaccine (TBV) candidates and drug molecules have been showed to limit the transmission of *Plasmodium*. Such strategies require good knowledge of the transmissible parasite stages biology in the field. In this purpose, a four year longitudinal *Plasmodium* prospection was investigated in the young children population of rural endemic area in Burkina Faso (West Africa), for *Plasmodium* infection. Malaria parasitogical tests were performed for any "healthy" child, and parasite density was quantified for each infected case. A total of 19,500 microscopic tests performed in 2000 children population showed *Plasmodium* infection in the children population at each month of the year. Analysis revealed more than 40% and 8% of asexual parasite and gametocyte infections respectively, per year. For 1599 gametocyte infections, ranged from 8 to 9256 gametocytes/µl of blood, an average of 55.20 gametocytes/µl (95% CI [45.79; 64.61]) was found. Regarding these densities the efficacy of two TBVs candidates (Pfs25 and Pfs230), as shown in laboratory in Burkina Faso, might be sufficient to limit *Plasmodium* transmission. Furthermore, following the temporal variation, the lower gametocyte density (33.10 gametocytes/µl 95% CI [22.72; 43.48]) observed from February to April suggest this period to be appropriated for any transmission blocking interventions.

929

EVALUATION OF TRANSMISSION RESERVOIR AND FIELD FEEDING ASSAYS TO INFORM TRANSMISSION BLOCKING VACCINE FIELD TRIAL DESIGN

Yimin Wu[1], Sara Healy[1], Michael P. Fay[1], Mahamadoun H. Assadou[2], Ruth Ellis[1], Ibrahima Baber[2], Agnes Guindo[2], Bronner Gongalves[1], Olga Muratova[1], Charles Luswata[1], Rathy Mohan[1], Mamadou Coulibaly[2], Issaka Sagara[2], Sekou Traore[2], Ogobara Doumbo[2], Patrick Duffy[1]

[1]National Institutes of Health, Rockville, MD, United States, [2]Malaria Research and Training Center, USTTB, Bamako, Mali

A transmission blocking vaccine (TBV) could be an integral part of malaria elimination and eradication. In preparation for a Phase 1 trial to test a Pfs25-based TBV malaria vaccine candidate in the target population, we surveyed gametocyte carriage rates by microscopy reading of thick smears and by quantitative PCR in Bancoumana, Mali. Starting jn June 2011, a total of 500 volunteers, from 3 months to 50 years of age, were recruited for monthly surveys of asexual and sexual parasite carriage. The carriage rates were higher among volunteers living closer to river and rice fields. Although the carriage rates were higher (12%) in age groups of 5-17 years old, typically 9% of adults older than 18 years of age carry gametocytes during the high transmission season. These adults are infectious to mosquitoes, as shown by various artificial feeding methods including Direct Skin Feeds (DSF) and Direct Membrane Feeds (DMF). Baseline mosquito infectivity was higher by DSF, followed by DMF with serum replacement. Smear reading by well-trained microscopists proved to be a very effective way to identify gametocyte carriers infective to mosquitoes. Based on these data, a Phase 1 study has been designed and planned to evaluate safety, immunogenicity, and transmission blocking activity induced by the Pfs25-EPA/Alhydrogel vaccine candidate product in adults in Bancoumana, Mali.

930

ANTIBODIES AGAINST A YEAST EXPRESSED PFS230 PROTEIN PRODUCED BY SCALABLE BIOPROCESSES BLOCK MOSQUITO TRANSMISSION OF MALARIA PARASITES AND FUNCTION TOGETHER WITH ANTIBODIES AGAINST PFS25

Nicholas MacDonald[1], Vu Nguyen[1], Richard Shimp, Jr.[1], Karine Reiter[1], Raul Herrera[1], Martin Burkhardt[1], Olga Muratova[1], Krishan Kumar[1], Joan Aebig[1], Kelly Rausch[1], Lynn Lambert[1], Nikiah Dawson[1], Darrick Carter[2], Randall F. Howard[2], Michael Fay[3], Steven G. Reed[2], Patrick E. Duffy[1], Yimin Wu[1], **David L. Narum**[1]

[1]Laboratory of Malaria Immunology and Vaccinology, National Institutes of Health, Rockville, MD, United States, [2]Infectious Disease Research Institute, Seattle, WA, United States, [3]Biostatistics Research Branch, National Institutes of Health, Rockville, MD, United States

Development of a *Plasmodium falciparum* transmission blocking vaccine (TBV) has the potential to markedly impact malaria control efforts. Pfs25-EPA, a leading TBV candidate is a chemically conjugated nanoparticle adsorbed on Alhydrogel®. In a phase 1 safety and immunogenicity trial, Pfs25-EPA/Alhydrogel® appeared safe and induced antibodies that partially blocked mosquito infectivity using an *ex vivo* feeding assay (SMFA). However, the level of reduction in transmission blocking activity appears insufficient in its current formulation. Efforts to evaluate another promising TBV candidate in the clinic, identified as the 230 kDa sexual stage protein Pfs230, have been thwarted for nearly a quarter of a century due to an inability to produce clinical grade protein containing the unique 6 cysteine-rich motif. Here, we report the scalable production of a recombinant subdomain of Pfs230, specifically Pfs230 domain 1 (D1) using a modified *Pichia pastoris* host. The secreted and purified recombinant Pfs230D1 protein has been fully characterized biochemically and biophysically. Antibodies against Pfs230D1 alone blocked transmission of malaria parasites and also had blocking effects when used in the presence of anti-Pfs25 antibodies by SMFA. Pfs230D1 and Pfs25 formulated in GLA-SE, the synthetic Toll-like receptor 4 agonist Glucopyranosyl Lipid Adjuvant in Stable Emulsion induced antibodies in *Aotus* monkeys that blocked mosquito transmission of malaria. The 6 cysteine-rich domain of Pfs230D1 is completely conserved among 200 parasite isolates. The combination of Pfs230D1 and Pfs25 in the form of chemically conjugated nanoparticles is a promising next step toward development of a vaccine that blocks malaria transmission.

931

RECOMBINANT PVS48/45 ANTIGEN EXPRESSED IN *E. COLI* GENERATES ANTIBODIES THAT BLOCK MALARIA THE TRANSMISSION IN *ANOPHELES ALBIMANUS* MOSQUITOES

Andres Vallejo[1], Yesid Solarte[2], Catherin Marin[1], Sócrates Herrera[1], Myriam Arévalo-Herrera[3]

[1]Caucaseco Scientific Research Center and Malaria Vaccine and Drug Development Center (MVDC), Cali, Colombia, [2]School of Health, Universidad del valle, Cali, Colombia, [3]Caucaseco Scientific Research Center and School of Health, Universidad del valle, Cali, Colombia

Transmission of malaria parasites from humans to *Anopheles* mosquitoes can be inhibited by specific antibodies to surface gametocytes proteins elicited during human malaria infection, these proteins are therefore considered to have potential for vaccine development. *Plasmodium vivax* Pvs48/45 is considered a promising gametocyte membrane surface antigen due to its potential functional role during parasite fertilization. The protein belongs to the 6-cy domain family characterized by its structural complexity what makes difficult to obtain high yields of soluble and properly folded protein when expressed as recombinant product. We

describe the expression of Pvs48/45 in *E. coli* and its biochemical and immunological characterization. The ~54kDa affinity-purified protein was reactive with sera (n= 57) of individuals from malaria endemic areas of Colombia and induced high ELISA antibody titers in mice and in Aotus monkeys when injected emulsified in Montanide ISA-51(. Antibodies from both mice and primates recognized the native parasite protein by Western blot and IFA (mean titer= 1:10,240) and efficiently blocked (>90%) the infection of *Anopheles albimanus* mosquitoes in membrane feeding assays. These results indicate that rPvs48/45 protein expressed in *E. coli* has the proper conformation able to induce antibodies that block sporozoite fertilization in the mosquito vector and therefore could be used as potential target to design a TB vaccine against *P. vivax*. Ongoing studies on the optimization and further protein characterization as well as immunogenicity and TB efficacy studies in primates will be presented.

932

A PHASE IA CLINICAL TRIAL OF A BLOOD-STAGE *PLASMODIUM VIVAX* VACCINE

Ruth O. Payne[1], Simone C. de Cassan[1], Kathryn H. Milne[1], Sean C. Elias[1], Anna L. Goodman[1], David Llewellyn[1], Nick J. Edwards[1], Ahmad Rushdi Shakri[2], Chetan E. Chitnis[2], Alfredo Nicosia[3], Sarah Moyle[4], Eleanor Berrie[4], Alison M. Lawrie[1], Adrian V. Hill[1], Simon J. Draper[1]

[1]University of Oxford, Oxford, United Kingdom, [2]International Centre for Genetic Engineering and Biotechnology, New Delhi, India, [3]Okairòs, Rome, Italy, [4]Clinical Biomanufacturing Facility, University of Oxford, Oxford, United Kingdom

Plasmodium vivax is the most geographically wide-spread malaria and is increasingly recognised as a significant cause of morbidity and economic loss, as well as occasional mortality. Control of *P. vivax* is challenging, with re-emergence in areas where it has previously been eliminated. Little progress has been made in development of a *P. vivax* vaccine with only two *P. vivax* antigens reaching Phase Ia clinical trials. Here we report on a Phase Ia clinical trial in Oxford using recombinant simian adenovirus ChAd63 and modified vaccinia virus Ankara (MVA) encoding the *P. vivax* antigen PvDBP (Duffy-binding protein region II) in a heterologous prime-boost regimen. Invasion of reticulocytes by *P. vivax* requires interaction between the parasite ligand Duffy-binding protein and its host receptor, the Duffy antigen receptor for chemokines (DARC). Unlike *P. falciparum* which can utilise multiple redundant pathways for erythrocyte invasion, the interaction between PvDBP and DARC is vital for *P. vivax*, making PvDBP a very promising antigen for vaccine development. Pre-clinical studies have shown that ChAd63 and MVA expressing PvDBP induce strong antibody immunogenicity and functional activity to block binding of PvDBP to DARC *in vitro*. This Phase Ia study of ChAd63-MVA PvDBP involves vaccinating 24 healthy volunteers in a dose escalation study. The dosing schedule involves ChAd63 PvDBP at 5 x 109 vp in the first four volunteers; ChAd63 PvDBP at 5 x 1010 vp in the next four volunteers; then two groups who will receive both ChAd63 PvDBP (5 x 1010 vp) followed by MVA PvDBP eight weeks later (at doses of 1 x 108 pfu - 2 x 108 pfu). Safety is the primary objective and safety reviews are undertaken throughout the study, particularly prior to dose escalation. The secondary objective is humoral and cellular immunogenicity which will be assessed using assays including PvDBP IFN-γ T cell ELISPOT, B cell ELISPOT, PvDBP IgG antibody ELISA and functional antibody analysis. This clinical trial is the first to be undertaken for a blood-stage antigen targeting *P. vivax* and will provide valuable insight into the utility of human vaccination against this well-described antigen.

933

IDENTIFYING CRYPTIC PROTECTIVE EPITOPES, GENERATING FULLY HUMAN MONOCLONAL ANTIBODIES AND APPLYING REVERSE VACCINOLOGY TO PROMOTE SUCCESSFUL MALARIA VACCINE DEVELOPMENT

Thomas L. Richie, Eileen F. Villasante, Sofia Casares

Naval Medical Research Center (U.S. Military Malaria Vaccine Program), Silver Spring, MD, United States

Several published studies indicate that in animal models of malaria, passive immunization with monoclonal antibodies targeting protective peptides contained within the sequence of leading malaria vaccine candidate antigens such as the circumsporozoite protein (CSP) provides much superior protection relative to active immunization with recombinant protein that includes the protective peptide, or with the protective peptide itself. While active immunization with recombinant protein or peptide induces overall antigen-specific antibody titers of equivalent magnitude to passive immunization with a monoclonal, as evidenced by sporozoite IFA or CSP ELISA, incubation with the protective epitope to absorb out peptide-specific activity does not appreciably affect the IFA titer of the actively-induced sera, indicating the presence of minimal antibody targeting the protective peptide. These observations suggest that evolutionary molding of protein architecture in *Plasmodium* due to continuous exposure to host immunity has resulted in antigens that are fundamentally poorly immunogenic for protective responses. In this presentation, these data will be reviewed and approaches will be proposed that could lead to effective passive immunization against malaria, or to the design of immunogens that, although potentially straying from native amino acid sequence, nevertheless induce protective responses in vaccine recipients. Key to these approaches is an emerging technology developed at the Naval Medical Research Center, US Military Malaria Vaccine Program, based on humanized mice, that can be used to rapidly generate fully human (and thus non-allergenic) monoclonal antibodies targeting malaria proteins. While manufacturing processes still require refinement, these reagents, likely both safe and amenable to repeated administration without loss of effectiveness, may gain importance for malaria prevention and treatment, and for regional campaigns for malaria elimination.

934

EFFECTS OF EARLY PARASITIC INFECTIONS ON CHILD GROWTH IN COASTAL KENYA

Monica Nayakwadi Singer[1], Indu Malhotra[2], Peter L. Mungai[3], Eric Muchiri[3], Ginny Gildengorin[1], Charles King[4], Christopher King[4], A. Desiree LaBeaud[1]

[1]Children's Hospital and Research Center, Oakland, CA, United States, [2]Case Western Reserve University Center for Global Health and Diseases, Cleveland, OH, United States, [3]Division of Vector Borne and Neglected Tropical Diseases, Ministry of Public Health and Sanitation, Nairobi, Kenya, [4]Center for Global Health and Diseases, Case Western Reserve University, Cleveland, OH, United States

Children in resource-limited tropical communities are heavily burdened by parasitic diseases that lead to high rates of morbidity and mortality. Few studies have examined at what age parasitic infections begin and what effects they have on early growth. Our objective was to determine the incidence and prevalence of parasitic infections (malaria, urinary schistosomiasis, soil-transmitted helminthes (STH), and lymphatic filariasis) in the first two years of life and examine their effects on growth. A 2005-2010 child cohort in Coastal Kenya was followed at birth and every 6 months until age 2 years. At each visit height, weight, and head circumference were measured and blood, stool, and urine were collected. Specimens were tested for presence of infection as follows: blood smear and PCR for malaria, Og4c3 antigen ELISA for filaria, Ritchie stool examination for STH, and SWAP IgG4 ELISA and urine filtration for *S. haematobium*. We computed descriptive statistics to estimate the infection rates. Mixed linear models were developed to determine the association

of each type of infection with growth variables.Of 182 children, 20 (11%) had at least one parasitic infection at 6 months, 32 (18%) at 12 months, 60 (33%) at 18 months, and 62 (34%) at 24 months. 56 (31%) of children had one infection and 14 (7.7%) were polyparasitized by the age of 2 years. All infections were documented as early as 6 months of age, except *Ascaris*. Infection prevalence increased with age. Malaria infection showed a statistically significant decrease in head circumference z score at 18 months (p=0.01). By 24 months, weight, height and head circumference z scores were decreased as compared to the uninfected group (p=0.05, 0.02, 0.01, respectively).Children with schistosomiasis during the first two years of life had a decrease in height z score (p=0.05). Children infected with STH had a significant decrease in head circumference z score by 24 months (p=0.05). *Strongyloides* infection was associated with a decrease in weight z score by 24 months (p=0.04). Parasitic infections begin early in infancy and increase in incidence in the first two years of life in Coastal Kenya. Malaria, schistosomiasis, and STH infections were associated with significant decreases in growth parameters by 24 months of age.

935

QUALITY OF CASE MANAGEMENT OF CHILDHOOD ILLNESSES PROVIDED BY HEALTH EXTENSION WORKERS IN ETHIOPIA

Nathan P. Miller[1], Agbessi Amouzou[1], Mengistu Tafesse[2], Elizabeth Hazel[1], Hailemariam Legesse[3], Tedbabe Degefie[3], Cesar G. Victora[4], Robert E. Black[1], Jennifer Bryce[1]

[1]Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, United States, [2]Alliance for Better Health, Addis Ababa, Ethiopia, [3]UNICEF Ethiopia, Addis Ababa, Ethiopia, [4]Federal University of Pelotas, Pelotas, Brazil

Ethiopia is scaling-up integrated community case management of common childhood illnesses (iCCM) in most regions of the country. Antibiotic therapy for childhood pneumonia and zinc for treatment of diarrhea have been added to the pre-existing management of malaria, diarrhea (with oral rehydration solution only) and malnutrition. We surveyed Health Extension Workers (HEWs) to assess the strength of iCCM implementation and the quality of care provided to sick children. This study was the first to evaluate the scale-up of iCCM in Ethiopia and the first rigorous assessment of quality of care provided by HEWs. It also adds to a limited evidence base on the quality of iCCM services provided by community-based health workers in sub-Saharan Africa. We conducted a cross-sectional survey in random samples of health posts in iCCM intervention and comparison areas in two zones of Oromia region. A total of 201 HEWs in 149 health posts were surveyed and 257 sick children were included. Data collectors trained in iCCM observed HEWs' consultations with sick children, carried out 'gold standard' re-examinations of children, conducted caretaker exit interviews, inspected iCCM commodities and patient registers and interviewed HEWs. Indicators of program strength, including availability of commodities and supervision, were significantly higher in intervention areas. HEWs in intervention areas provided correct treatment and/or referral for 64% of children with major iCCM illnesses (pneumonia, diarrhea, malaria, malnutrition and measles). The proportions of children correctly managed for pneumonia, diarrhea and malnutrition were 72%, 79% and 59%, respectively. Only 6% of children received an antibiotic when it was not indicated and no children received unnecessary antimalarials. However, only 34% (13/38) of children with severe illness were correctly managed and 54% (34/63) of children needing referral to a higher-level health facility were referred. Utilization of HEW case management services was low, with an average of only 16 sick child consultations per health post in the previous month in intervention areas. Implementation of the iCCM program in Jimma and West Hararghe zones was very strong and HEWs are generally providing high-quality case management services. However, for the program to have a significant impact on child mortality, management of severe illnesses and utilization of HEW services must be improved.

936

MATERNAL INFECTIONS AND ANEMIA IN INFANCY AND EARLY CHILDHOOD

Indu Malhotra[1], Mary A. Uyoga[2], Daniel J. Tisch[1], Arlene E. Dent[1], Penny A. Holding[2], Christopher L. King[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]International Centre for Behavioural Studies, Mombasa, Kenya

Iron deficiency (ID) is common in pregnant women and children worldwide. When ID occurs in infancy, neurological development can be impaired. Through transplacental iron transfer, infants acquire iron stores *in utero*. Factors such as chronic inflammation or profound ID in mothers can impair iron transport, thus increasing a child's ID risk. To assess risk factors for anemia in infancy and childhood, we studied offspring of Kenyan women living in nearby rural (N=303) and urban (N=418) areas, assessing hemoglobin (Hb) levels at birth and at 6, 10, and 14 weeks of age, and every 3 months thereafter until age 3. Forty-two percent of mothers residing in the rural area were anemic (Hb<9 gm/dl) at delivery, as compared to 4.7% of urban women; despite this difference, newborns in the rural and urban sites had similar mean Hb levels and frequencies of anemia (Hb<12.5 gm/dl,16.8% and 17.9%, respectively). Mean Hb in offspring remained similar between sites until after 10 weeks of age, when mean Hb diverged; children in the rural population had a 2-3 gm/dL lower mean Hb when compared to urban children, and the difference persisted until age 2 years. This finding could not be explained by sex, variation in feeding practices, rates of weight gain or maternal mean Hb level at the sites. Although maternal age and maternal BMI were associated with low mean Hb levels in offspring, these factors also did not explain differences in mean Hb. However, we found that maternal helminth and malaria infections during pregnancy had been documented in over 60% of the rural women, while in urban women the frequency was <10%. Whether these women developed anemia of chronic inflammation and/or ID is under investigation. We suggest that women with chronic parasitic infections develop anemia of chronic inflammation, impairing transplacental transport of iron during pregnancy. This could result in inadequate iron stores in offspring and insufficient support of erythropoiesis at 10 weeks of age. The importance of treating chronic parasitic infections during pregnancy should be emphasized.

937

IMPACT OF AN INTENSIVE PERINATAL HANDWASHING PROMOTION INTERVENTION ON MATERNAL HANDWASHING BEHAVIOR IN THE NEONATAL PERIOD: FINDINGS FROM A RANDOMIZED CONTROLLED TRIAL IN RURAL BANGLADESH

Pavani Kalluri Ram[1], Sharifa Nasreen[2], Kelly Kamm[1], Jelena Vujcic[1], Swapna Kumar[1], Md. Anisur Rahman[2], K. Zaman[2], Shams El Arifeen[2], Stephen P. Luby[3]

[1]University at Buffalo, Buffalo, NY, United States, [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [3]Stanford University, Palo Alto, CA, United States

One-quarter of neonatal deaths are attributed to infections, including sepsis and pneumonia. Maternal handwashing with soap prevents pneumonia among young children, and may prevent neonatal sepsis. We examined the impact of an intensive handwashing promotion implemented in the perinatal period on the handwashing behavior of mothers of neonates. We conducted a randomized controlled trial enrolling pregnant women at 28-32 weeks gestation in Matlab, Bangladesh. After collecting baseline data, we randomized participants to intensive handwashing promotion or control. Both study arms received maternal and neonatal care counseling and a clean delivery kit. In 1-2 antenatal visits, and 2 post-natal visits, we used a participatory approach to motivate maternal handwashing with soap, promoting handwashing as a nurturing behavior, among the intervention arm; soap and water containers were provided for handwashing in the neonate's sleeping room,

the courtyard, and outside the latrine. In both study arms, we observed presence of soap and water at handwashing places during Weeks 1, 2, and 4 after birth. Using data from 3-hour structured observations in Week 4, we estimated intervention impact on maternal handwashing overall and at possible times of pathogen transmission. In total, 125 women were randomized to intervention and 124 to control. During the neonatal period, soap and water were present more frequently at a handwashing place in intervention homes than controls: Week 1 (84% vs. 45%); Week 2 (69% vs. 35%); Week 4 (69% vs. 27%). In Week 4, observed handwashing overall was 40% higher among intervention mothers than controls (p<.001). Although soap was observed at a minority of pathogen transmission times in both intervention (8%) and control (2%) groups, intervention mothers washed hands with soap 4.1 times as frequently as controls (p<.0001). Intensive promotion of handwashing with soap, and provision of needed materials, in the perinatal period resulted in increased availability of soap and water at designated handwashing places, and a four-fold increase in maternal handwashing with soap. Although handwashing with soap was infrequent at possible times of pathogen transmission, the overall increase in handwashing with soap may be sufficient to reduce neonatal infections.

938

HOME-BASED MANAGEMENT OF MALARIA, DIARRHEA AND ACUTE RESPIRATORY ILLNESS IN CHILDREN: THE EXPERIENCE IN SENEGAL

Seynabou Gaye[1], Fatou Ba[1], Alioune Badara Gueye[1], Moussa Ndour[1], Ibrahima Diallo[1], Medoune Ndiop[1], Aida Gadiaga[2], Agnes Ndiaye[2], Aissatou Diop[2], Mamadou Moustapha Diaw[3], Mady Ba[1]

[1]National Malaria Control Programme, Dakar, Senegal, [2]Division of Food and Nutrition Child Survival, Dakar, Senegal, [3]Centre for Educational and Vocational Guidance, Diourbel, Senegal

In 2008, the Senegal National Malaria Control Program (NMCP) introduced home-based management of malaria (PECADOM), with excellent results. Evaluations of the program recommended including diarrhea and acute respiratory illness (ARI) management in the package of interventions to increase impact on all cause under five mortality. The NMCP and partners conducted a pilot of PECADOM integrating diarrhea and ARI management in 87 villages in five districts. A volunteer home care provider (DSDOM) was selected from each of the communities and participated in four days of classroom training and a 15 day practicum. After training, an installation ceremony was held for each DSDOM, and each received a medical supply box with a timer, rapid diagnostic tests (RDT), artemisinin-based combination therapy (ACT), oral rehydration salts, zinc, and co-trimoxazole. All patients were recorded in a register. Monitoring included supervision, monthly coordination meetings and biannual reviews including all involved in the implementation of integrated PECADOM at all levels. Data were obtained from registers, supervision visits, and the biannual review. Interviews were conducted with members of the community. A total of 87 DSDOMs were trained and installed. During six months, 3,177 children under five years were seen in consultation by DSDOMs. Of these, 13% had fever and received an RDT, 18% had a cough and runny nose, 31% had uncomplicated pneumonia, and 29% had diarrhea. There were 173 confirmed cases of malaria, all of which were reported treated and cured. On average, 10% of cases seen in consultation by DSDOMs were referred to health posts, the majority of which were cases with negative RDT. Communities were actively involved and welcomed the implementation of the strategy in their village. The DSDOMs expressed a sense of purpose and respect in their community. The encouraging results achieved after six months of implementation of the pilot suggest the need to scale up to ensure early and universal access to quality case management of malaria, diarrhea and ARI. Integrated PECADOM is an important tool to ensure equity in access to care and decrease all cause under five mortality.

939

VARIATION IN THE QUALITY AND COST OF TREATMENT FOR CHILDHOOD DIARRHEA, MALARIA AND PNEUMONIA: COMMUNITY AND FACILITY BASED CARE IN RURAL UGANDA

Seyi Soremekun[1], Frida Kasteng[1], Raghu Lingam[1], Guus ten Asbroek[1], Anna Vassall[1], Betty Kirkwood[1], Agnes Nanyonjo[2], Edmound Kertho[2], Karin Kallander[3], inSCALE Study Group[4]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Malaria Consortium Uganda, Kampala, Uganda, [3]Malaria Consortium Africa, Kampala, Uganda, [4]The inSCALE Study Group consists of: Sylvia Meek, James Tibenderana, Patrick Lumumba and Maureen Nakirunda (Malaria Consortium, Kampala, Uganda); Zelee Hill and Daniel Strachan (University College London, London, United Kingdom), Uganda, United Kingdom

Diarrhoea, malaria and pneumonia together account for 36% of deaths in children under 5yrs of age, yet facility-based treatment is often inaccessible or inadequate in low income/rural settings. Integrated community case management (iCCM) has been recommended by the WHO/UNICEF as an effective means of tackling the treatment gap through the training and deployment of community health workers (CHWs). The inSCALE study aims to improve the motivation and performance of CHWs and thus increase coverage of appropriately treated children in Uganda and Mozambique. As part of a cross-sectional survey of 6553 randomly sampled households in villages with iCCM CHWs in western Uganda, we identified 3900 households with 6501 children aged 2-59months. 11%, 47% and 24% of study children had recently had episodes of diarrhoea, fever and pneumonia (DFP) respectively. Only 54% of children were given appropriate antibiotics for pneumonia, 47% with fever were given ACTs, rising to 73% in malaria-confirmed cases, and 30% received ORS for diarrhoea. Coverage of ORS+zinc treatment for diarrhoea was low at 9% as was blood testing (RDTs or microscopy) in cases of fever (27%). Relatives who sought care for sick children accessed CHWs in 22% of cases, whilst the majority visited health facilities (63%) the largest proportion of which were private facilities or doctors (38%). However the chances of receiving correct treatment for DFP was lowest in the private sector (23%, 32% and 56% respectively) and highest via CHWs (53%, 71% and 61% respectively) which were also the least expensive option at $0/visit, compared to $2/visit in the private sector. There was a small but consistent trend of reduced access to appropriate treatment in the poorest 40% of households vs. the richest 40% (coverage differences -6%, -6% and -10% for DFP respectively). iCCM has great potential to reduce coverage gaps in the treatment of community-acquired infections at low cost to families. CHWs were accessed in a small number of cases of DFP; this is likely to increase over time with the embedding of the national iCCM policy.

940

OUTBREAK OF SUDDEN DEATHS AMONG CHILDREN LIVING NEAR LYCHEE ORCHARDS IN NORTHERN BANGLADESH

Md. S. Islam[1], Ahmed Raihan Sharif[2], Hossain M. Sazzad[1], A. K. Dawlat Khan[1], S. M. Murshid Hasan[1], Shirina Akter[1], Golam D. Harun[1], Stephen P. Luby[1], James Heffelfinger[3], Mahmudur Rahman[4], Emily S. Gurley[1]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [2]Institute of Epidemiology, Disease Control and Research, Dhaka, Bangladesh, [3]Global Disease Detection Program, Centers for Disease Control and Prevention, Atlanta, Georgia, USA, Dhaka, Bangladesh, [4]Institute of Epidemiology, Disease Control and Research, Dhaka, Bangladesh

In June 2012, a cluster of sudden deaths among children aged 2-10 years was reported from northern Bangladesh. In response, a team from the Government of Bangladesh's Institute of Epidemiology, Disease Control and Research and icddr,b conducted an investigation to describe clinical

and exposure histories of cases to generate hypotheses about possible causes of illness; the minimum criterion for classification as a suspected case was presence of convulsion. We reviewed hospital records to identify cases and interviewed family caregivers about the symptoms and exposures the case had. Since many case-households were located adjacent to lychee orchards, we collected information on the types of pesticides used in the area and case-patient exposures to the orchards and lychees. Fourteen children met the case definition; 13 (93%) died and the median time from illness onset to death was 20 hours. For 64% of cases, the illness started with a sudden outcry in the early morning followed within hours by convulsions and unconsciousness. The most common signs were convulsions (100%), unconsciousness (86%), frothy discharge from the mouth (86%), altered mental status (71%) and fever (70%). Four children had mid-dilated or fixed pupils and six had lung crepitations on auscultation. In the 24 hours before illness onset, all of the cases had either visited lychee orchards (n=11) or consumed lychees (n=7) where multiple pesticides including insecticides, fungicides and other chemicals were used at least three times during the preceding two weeks. Eight case-households bordered lychee orchards, and five case-households were located within approximately 100 meters of a lychee orchard. Caregivers reported that many cases peeled lychee fruits with their teeth and ate unwashed lychees. The clinical manifestations and course of illness of cases suggest that this outbreak was due to poisoning, likely from pesticides used in nearby lychee orchards. Close proximity of the case-households to lychee orchards, heavy use of pesticides in the orchards, and the children's exposure to lychee orchards and fruit increased their exposure to agricultural chemicals. Interventions are needed to limit children's exposures to dangerous agricultural chemicals pesticides. In South Asia, repeated outbreaks of child encephalitis during lychee harvesting season have been reported in with undetermined etiology which could be similar to what we observed in Bangladesh.

941

USE OF ANTHROPOGENIC MOSQUITOES FOR SURVEILLANCE OF BLOOD-BORNE PATHOGENS

Nathan D. Grubaugh, Massamba Sylla, Thomas Harrison, Benjamin J. Krajacich, Gregory D. Ebel, Brian D. Foy, Doug E. Brackney

Colorado State University, Fort Collins, CO, United States

Biosurveillance is the process of collecting, analyzing, and interpreting biosphere data in order to provide early detection of biological threats to human health. Obtaining meaningful samples for surveillance, particularly human blood, has limited pathogen detection in many underdeveloped countries. Therefore, we explored the possibility of exploiting the intrinsic host-feeding preferences and behaviors of Anopheline mosquitoes to enhance biosurveillance efforts. Earlier studies have demonstrated the technical feasibility of this approach. Specifically, human papillomavirus was detected in field collected mosquito pools during a metagenomics analysis of the mosquito virome. In addition, highly pathogenic avian influenza virus was detected in engorged mosquitoes from a poultry farm in Thailand by RT-PCR. In this study we aimed to define the limitations of this approach in the laboratory and determine its potential application in the field. We hypothesize that blood-fed mosquitoes can be used in surveillance and discovery of pathogens in underdeveloped countries. To test this, colonized *Anopheles gambiae* mosquitoes were fed on blood containing serial dilutions of chikungunya, influenza A, West Nile, Pirital, and human immunodeficiency-1 viruses. Twelve hours later the contents of the mosquito midguts were pressed onto filter paper to inactivate the virus and preserve the nucleic acids. The limits of viral RNA detection by RT-PCR were between 10 and 100 genome equivalents per microliter of fed virus. Additionally, we could differentiate between human, dog, cow, and sheep blood meals using multiplexed PCR. The samples will be further assessed for detection limits using deep sequencing. In August of 2012 our pathogen surveillance system was evaluated using blood fed *An.* mosquitoes collected at dawn from homes in Bandafassi, Senegal. Preliminary deep sequencing results of the mosquito blood meals revealed the presence of RNA homologous to herpes simplex virus type 2 and several *Plasmodium* species. We have demonstrated that this is a creative solution to overcome the technical and logistical obstacles, such as training local health professionals and maintaining sample cold-chain, for acquiring human blood for pathogen surveillance.

942

BIODISTRIBUTION AND TRAFFICKING OF NANOPARTICLES IN *ANOPHELES GAMBIAE* ADULT AND LARVAL MOSQUITOES

Barry Beaty[1], Cynthia Paquette[1], Jonathan Carlson[1], Lyric Bartholomay[2], Paul Airs[2], Yashdeep Phanse[2], Joseph D. DeSimone[3], Chris Luft[3], Jillian Perry[3], Jing Xu[3]

[1]Colorado State University, Fort Collins, CO, United States, [2]Iowa State University, Ames, IA, United States, [3]University of North Carolina, Chapel Hill, NC, United States

There is a public health imperative to develop new chemical insecticides with new modes of action for vector control. Emerging resistance to pyrethroids, the principal active ingredients (AIs) in bednets and in indoor residual and space spraying, is of immense concern. We are developing an innovative, flexible and adaptable, molecular mosquitocide (MM) approach to control disease vectors. A MM is comprised of 1) an "active sequence" (AS) dsRNA to induce an RNAi response to silence a targeted mosquito gene and 2) a nanoparticle delivery system that promotes environmental stability of and efficacious delivery of the AS to vector target organs and cells to induce a systemic RNAi response resulting in vector lethality. This approach exploits the explosion of information accrued in mosquito genetics and genomics to provide an almost unlimited number of potential target genes and sequences for RNAi-based MMs. Critical to the development of MMs is selection of optimal nanoparticles for delivery of the ASs. Nanoparticles are produced using state of the art Particle Replication in Non-wetting Templates technology. Using PRINT^{R} Platform technology nanoparticles of defined size, shape, aspect ratio, surface charge and modulus have been fabricated and compared in their biodistribution in adults and larvae following *per os* challenge and intrathoracic injection. Nanoparticles do differ dramatically in their biodistribution and cellular internalization potential depending upon such characteristics as size, aspect ratio, and charge. Studies to determine optimal particle characteristics for delivery of ASs (approximately 400 bp dsRNAs) to selected programmed cell death genes to induce a lethal, systemic RNAi response are in progress. MMs offer exciting potential to expand the armamentarium for vector control beyond the MOAs of conventional chemical insecticides and to potentially provide a flexible platform approach for delivering new and improved ASs to vectors in response to the emergence of resistance.

943

LADIES FIRST: EFFECT OF GENDER ON BITING RISK BY TSETSE FLIES

Vanja Kovacic[1], Inaki Tirados[1], Johan Esterhuizen[1], Clement T. Mangwiro[2], Michael J. Lehane[1], Steven J. Torr[1]

[1]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [2]Bindura University of Science Education, Harare, Zimbabwe

There is renewed vigour in efforts to eliminate sleeping sickness (human African trypanosomiasis; HAT) caused by subspecies of *Trypanosoma brucei* transmitted by tsetse flies. A better understanding of risk factors for HAT would contribute to this effort. Towards this goal, we carried out studies in the West Nile region of Uganda to assess how traditional gender roles influence exposure to tsetse. Sixty participants (thirty women and thirty men) from randomly selected households located in historical Gambian HAT foci wore a small global positioning system (GPS) during the course of their usual daily activities. Participants were also interviewed about their daily routines. GPS data were analysed using a geographical information system (GIS) and transcribed interviews were analysed using thematic analysis. Gender-specific tasks related to fetching water and gathering

firewood meant that the number and duration of visits to river banks and forested areas, where tsetse are more abundant, was greater for women than men. Women were also more likely to visit these risk zones during times of day when tsetse are active. Farming activities undertaken by men and women also increased exposure to tsetse, especially during the dry season when planting of crops and herding of livestock is concentrated in riverine areas. Overall, we estimate that women were more than twice as likely to visit tsetse-infested habitats as men. The implications of our results for the transmission and control of HAT are discussed. We conclude that strategies specifically directed at reducing risk of sleeping sickness for women are needed.

944

EXAMINATION OF THE DEVELOPMENTAL NEUROGENETIC BASIS OF SEXUAL DIMORPHISM IN *AEDES AEGYPTI*

Michael Tomchaney[1], Keshava Mysore[2], Molly Duman-Scheel[2]

[1]University of Notre Dame, Notre Dame, IN, United States, [2]Indiana University School of Medicine at Notre Dame, South Bend, IN, United States

Most animal species exhibit sexually dimorphic behaviors, many of which are linked to reproduction. A number of these behaviors, including blood feeding in female mosquitoes, contribute to the global spread of vector-borne illnesses. However, knowledge concerning the extent of sexual dimorphisms in the structure of the central nervous system, the control of sex-specific behaviors by sexually dimorphic neurons, and the developmental genetic basis for sexually dimorphic behavior is limited in any organism, including mosquitoes. In this investigation, we used custom microarrays to examine global differences in male vs. female gene expression in the developing brain of the dengue and yellow fever vector mosquito *Aedes aegypti.* The array uncovered 2,528 statistically significant differentially expressed genes. Genes upregulated in females were predominantly implicated in proteolytic and metabolic gene ontology (GO) processes. Genes upregulated in males were often associated with metabolic GO terms, including polysaccharide metabolism. Genes which have been implicated in behavior, including feeding, were also dimorphically expressed. A number of differentially expressed genes were also linked to critical developmental signaling pathways, including the Wnt and Hedgehog signal transduction cascades. Genes from these metabolic, behavioral, and developmental pathways were prioritized for whole-mount gene expression analyses in the pupal brain. These expression studies validated the microarray results and allowed for identification of sexually dimorphic regions in the pupal brain. In many cases, dimorphic gene expression localized to the optic lobe. We are now pursuing siRNA-mediated knockdown studies to functionally examine the roles of dimorphically expressed genes. These studies are providing insight into the genetic differences underlying sexually dimorphic neural circuitries and behaviors that promote the spread of disease.

945

COORDINATE REGULATION OF ODORANT RECEPTOR EXPRESSION AND OLFACTORY RECEPTOR NEURON TARGETING IN *AEDES AEGYPTI* BY THE TRANSCRIPTION FACTOR SINGLE-MINDED

Keshava Mysore, Molly Duman-Scheel

Indiana University School of Medicine at Notre Dame, South Bend, IN, United States

Knowledge of the mechanism that specifies mosquito olfactory receptor neurons (ORNs) to express a particular odorant receptor (OR) from a large OR pool, an important step for odor detection and discrimination, is lacking. Here, we investigate this process in *Aedes aegypti*, the dengue and yellow fever vector mosquito. Studies in other organisms have suggested that the combination and levels of expression of various cis-regulators of transcription in ORNs generates the OR regulatory matrix, a code governing which particular OR gene is expressed and which are repressed in any given ORN. Mosquito OR expression is likely to be regulated in a comparable manner. We have identified eight transcription factors (TFs) which are expressed in the developing antennae that are hypothesized to function in the Ae. *aegypti* OR regulatory matrix. Each TF is expressed in a subset of *Ae. aegypti* antennal ORNs, and expression levels of each TF varies from neuron to neuron within this subset. Searches for consensus binding site sequences for the TFs uncovered multiple binding sites residing in the 5′ flanking sequences and first introns of multiple OR genes. The transcription factor Single-minded (Sim) has binding sites in ~50% of the OR genes, suggesting that it may function as a major regulator of OR expression. To functionally test this hypothesis, we used chitosan/siRNA nanoparticles to target sim during olfactory development. These experiments demonstrated that Sim regulates expression of a subset of OR genes and functions in the *Ae. aegypti* OR regulatory matrix. These findings correlated with an odorant tracking behavioral defect. Interestingly, *sim* knockdown animals also displayed severe antennal lobe defects, including improper ORN targeting and projection neuron defects coincident with a collapse in the structure and shape of the antennal lobe and individual glomeruli. The results of this investigation demonstrate that Sim functions in the coordinate regulation of OR expression and ORN targeting, two processes that dictate what odors will be detected by a neuron and which behaviors are elicited in response to these odors.

946

MALE-FEMALE MOLECULAR INTERACTIONS SHAPING THE REPRODUCTIVE SUCCESS OF *ANOPHELES GAMBIAE* MOSQUITOES

Flaminia Catteruccia[1], Francesco Baldini[1], Paolo Gabrieli[2], Aurelio Serrao[2], Sara Mitchell[3], Evodxia Kakani[1], Francesca Mancini[2]

[1]Harvard School of Public Health, Boston, MA, United States, [2]University of Perugia, Terni, Italy, [3]Imperial College London, London, United Kingdom

The high reproductive rate of *Anopheles gambiae* mosquitoes is one of the principal components of their competence as malaria vectors, and is a target for novel vector control strategies aimed at reducing malaria transmission. Important biological processes underlying fecundity and fertility rates of *An. gambiae* mosquitoes are started by the interactions between male seminal fluid transferred during mating and female molecular pathways modulated by copulation. These yet unknown interactions trigger major changes in the physiology and behavior of females, including a reduced receptivity to further copulation, an increased egg production and the induction of egg laying. Here we identify a cascade of molecular events triggered by mating that increases egg development and facilitates egg laying in *An. gambiae* females. Using a combination of high-throughput studies followed by in depth functional analyses, we have identified a female Mating-Induced Stimulator of Oogenesis (MISO) that regulates the increase in egg development observed after mating and blood feeding. We also determine that MISO is regulated by and interacts with male factors transferred during copulation, and that this interaction is required for the correct accumulation of lipids in the developing oocytes. Moreover, by preventing the coagulation of seminal secretions in the male and their transfer to the female, we present evidence of the mechanisms inducing oviposition in these mosquitoes. These findings increase our knowledge of biological processes important for mosquito reproduction, and reveal possible targets for the design of novel tools for the control of natural vector populations.

947

THE VIRULENCE OF CHAGAS DISEASE AGENT *TRYPANOSOMA CRUZI* IN ITS INSECT VECTOR

Jennifer K. Peterson[1], Andrea L. Graham[1], Andrew P. Dobson[1], Omar Triana[2]

[1]Princeton University, Princeton, NJ, United States, [2]Universidad de Antioquia, Medellin, Colombia

Avoiding the over-exploitation of the resources in a food patch while still obtaining all the resources needed to live and reproduce is a common challenge among all creatures. For many parasites that live inside another organism this is especially tricky because they do not have the option of moving to a new patch should they be too virulent and overexploit their current one. The classic idea is that the avoidance of host death should favor intermediate levels of parasite virulence, however it is known that this is not the case: parasites actually exhibit a wide range of virulence. In our work we investigate parasite co-infection and strain differences as drivers of virulence variation in Chagas disease vector *Rhodnius prolixus* and two of its parasites *Trypanosoma cruzi* and *T. rangeli*. We found that *T. cruzi-T. rangeli* co-infection significantly reduces the survival of *R. prolixus* up to 30 days post-infection, indicating that this co-infection could be removing *T. cruzi*-infected vectors from the Chagas disease transmission cycle before it is transmitted. We have also found that *T. cruzi* virulence in *R. prolixus* is highly variable, with *R. prolixus* death ranging from 2-80% depending on *T. cruzi* strain (all DTU I). Our aim is to provide insights into the ecology of the Chagas disease transmission cycle and ultimately contribute to strengthen Chagas disease prevention efforts.

948

VERTICAL TRANSMISSION BIOLOGY AND LOCALIZATION OF *NEORICKETTSIA RISTICII* IN LIFE CYCLE STAGES OF THE DIGENEAN *PLAGIORCHIS ELEGANS*

Stephen E. Greiman, Vasyl V. Tkach, Jefferson A. Vaughan

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

Neorickettsia are obligate intracellular bacterial endosymbionts of digeneans. These endosymbionts pass through all stages of digenean life cycles via vertical transmission. They may also be passed from digeneans to vertebrates via horizontal transmission and cause diseases such as Sennetsu fever in humans, Potomac horse fever and salmon dog poisoning. Despite the role of these bacteria as pathogens in humans, domestic animals and wildlife, there is much to learn about their diversity, host associations and fundamental biology. Practically nothing is known about the quantitative aspects of their transmission or their distribution in digenean host tissues. We have screened digenean cercariae from snails collected in eastern North Dakota for the presence of *Neorickettsia* using real time PCR. We found two digenean species infected with *Neorickettsia*: *Plagiorchis elegans* with *N. risticii* and *Diplostomum sp.* with *Neorickettsia sp.* We have established the first laboratory experimental model of *Neorickettsia* using cercariae of *P. elegans* from the nature as a starting point. We were able to sustain *Neorickettsia* throughout several complete life cycles of *P. elegans*. The system included aquatic snails, *Lymnaea stagnalis,* as the first intermediate host, mosquitoes, *Culex pipiens,* as the second intermediate host and hamsters, *Mesocricetus auratus,* as the definitive host. The experimental model allowed us to quantify the efficiency of vertical transmission through the digenean life cycle (using real time PCR) and study the details of *Neorickettsia* localization in all developmental stages of digeneans (using immunofluorescent microscopy). Vertical transmission efficiency of *N. risticii* was invariably lower than 100% in cercariae and eggs, but reached 100% in sporocysts. The localization of *N. risticii* in organs and tissues of digeneans provides explanations for the differences in the infection rates among different stages.

949

THE DYNAMIC NATURE OF THE *SCHISTOSOMA MANSONI* APICAL TEGUMENT MEMBRANE IS DEPENDENT ON CYTOPLASMIC MOTOR PROTEINS

Leigh Schulte[1], Jason Mulvenna[1], Geoffrey Gobert[1], Malcolm Jones[2]

[1]Queensland Institute of Medical Research, Brisbane, Australia, [2]School of Veterinary Science, The University of Queensland, Brisbane, Australia

The tegument of *Schistosoma mansoni* serves a variety of critical biological roles for the parasite, including nutrition, osmoregulation, immune evasion and immuno-modulation. Dynein light chains (DLCs) and tetraspanins (TSPs) are prominent proteins in the tegument and appear to be intimately linked with other molecules present in the apical, host-interactive, membrane of the parasites. In other eukaryotic cells, cytoplasmic DLCs are a subunit of motor complexes involved in diverse cellular translocation events as protein trafficking, mitosis and ciliary beating. DLCs of the schistosome tegument likely have similar roles and are likely to be important in the development and renewal of the tegument membrane, thereby contributing to parasite survival in its host. TSPs are membrane-spanning proteins that act as scaffolds for the formation of membrane-associated protein complexes comprising a wide variety of proteins. These TSP-enriched microdomains are known to play a wide range of roles within human cells, including maintenance of cell morphology and cell signalling. As an anti-schistosomal vaccine SmTSP-2 has shown high protection, however its biological role in the tegument remains unknown. To explore protein interactions within the tegument we have used multiple methods, including Blue Native polyacrylamide gel electrophoresis and protein crosslinkers, coupled with in-line liquid chromatography-tandem mass spectrometry. To functionally characterise selected tegumental proteins, RNA interference has been be used to knock down the gene of interest, and phenotypic changes are visualised by electron microscopy. This study provides insights into the molecular complexes associated with the host-interactive surface of schistosomes and will highlight which molecules are associated with the surface membranes and how these molecules functionally contribute to the maintenance of the parasite host-parasite interface. Tegument development and maintenance are critical biological functions for the survival of the parasite within the mammalian host and this research may lead to the identification of novel targets for vaccination or drug therapy for schistosomiasis.

950

DEVELOPMENT OF SEROLOGICAL TECHNIQUES FOR THE DIAGNOSIS OF ZOONOTIC *SCHISTOSOMA JAPONICUM* INFECTION THROUGH THE USE OF RECOMBINANT PROTEINS

Jose Ma. M. Angeles[1], Yasuyuki Goto[2], Masashi Kirinoki[3], Lydia R. Leonardo[4], Masahito Asada[1], Elena A. Villacorte[4], Pilarita T. Rivera[4], Noboru Inoue[1], Yuichi Chigusa[3], Shin-ichiro Kawazu[1]

[1]Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan, [2]University of Tokyo, Tokyo, Japan, [3]Dokkyo Medical University, Tochigi, Japan, [4]University of the Philippines-Manila, Manila, Philippines

Schistosomiasis continues to be a public health problem in endemic countries. In the Philippines, schistosomiasis is endemic in 28 provinces caused by the zoonotic parasite *Schistosoma japonicum.* The role of animal reservoirs on the parasite's life cycle was not given much importance which might be the main hindrance in the possible elimination of the disease. Disease surveillance therefore should include both human and animals that might harbor and continually transmit the infection. In this study, we aim to develop a more sensitive and specific serological diagnosis for schistosomiasis both for human and animal host using recombinant proteins. Thioredoxin peroxidase-1 (SjTPx-1) and four tandem repeat proteins (Sj1TR, Sj2TR, Sj4TR, Sj7TR) using enzyme-linked immunosorbent assay (ELISA) were tested to determine their serological importance in human, water buffalo and dog diagnosis. Based on the results, SjTPx-1 might be used as a 'universal' diagnostic antigen that can detect human

and animal schistosome infection, whereas Sj7TR for both human and dog diagnosis and Sj1TR for water buffalo diagnosis. These results might encourage the development of a more accurate diagnostic test both for humans and animal schistosome infection based on the recombinant antigens and will therefore improve the disease surveillance leading to the possible elimination of this neglected parasitic disease in endemic countries like the Philippines.

951

EXPLORING SCHISTOSOMIASIS DIAGNOSIS USING MAGNETIC SEPARATION TECHNOLOGIES

Malcolm K. Jones[1], Stephan Karl[2], Renata R. Candido[3], Rafael Lucyk-Maurer[4], Lucia Gutierrez[2], Robert Woodward[2], Tim St Pierre[2], Carlos Graeff Teixeira[5]

[1]University of Queensland, Gatton, Australia, [2]University of Western Australia, Perth, Australia, [3]Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil, [4]Pontifícia Universidade Católica do Rio Grande do Sul, P, Brazil, [5]University of Queensland, Porto Alegre, Brazil

There is a great need for reliable and sensitive diagnostic tests for schistosomiasis that are affordable and able to be applied in field and laboratory settings. We have shown that magnetic beads bind strongly to schistosome eggs and can be used to manipulate and purify the eggs. The interaction between the beads and eggs is intriguing and of potential value in diagnosis. Adult schistosomes live in the vasculature of their human host, where they feed extensively on blood. Females are adapted to process large quantities of blood cells, which are digested using extracellular and epicellular digestive strategies. In addition to their requirements for catabolic products of haemoglobin, schistosomes also have nutritional dependence on many other metabolites and trace elements, notably iron (Fe). A number of pathways for Fe and haem uptake, transmembrane transport and storage have been identified for schistosomes. Schistosomes store abundant quantities of iron in vitelline glands, a follicular network of cells that synthesise precursors for eggshell formation. We have shown that this Fe store is most likely used during eggshell formation, becoming incorporated into the matrix and as iron-phosphate particles within pores of the shell. In this presentation, we will outline our studies on Fe and haem metabolism in schistosomes, focusing specifically on fate of Fe in embryogenesis. We will then discuss possible mechanisms for the strong interactions between magnetic beads and schistosome eggs and how this may be exploited for future diagnostic developments.

952

NEW GOLD STANDARD OF SCHISTOSOME DIAGNOSIS WITHOUT USING STOOL

Nilanjan Lodh[1], Jean M. Naples[1], Kwabena M. Bosompem[2], Clive J. Shiff[1]

[1]Johns Hopkins University, Baltimore, MD, United States, [2]Noguchi Memorial Institute for Medical Research, Legon, Accra, Ghana

Schistosomiasis is a worldwide communicable disease caused by several species of genus Schistosome can be easily transmitted to human host. Clinical diagnosis of two major human schistosomes, *Schistosoma mansoni* and *S. haematobium* lacks sensitivity and is cumbersome to conduct. So control strategies based on targeted mass drug administration (MDA) to succeed it is essential to have a simple, easy to operate, sensitive and accurate test. The standard diagnostic tests, including stool examination by Kato-Katz (KK), detection of egg (dipstick) and blood (haematuria) in urine lack sensitivity, especially in low endemic settings. We compared diagnostic efficacy of KK, haematuria and polymerase chain reaction (PCR) based on species specific DNA to detect *S. mansoni* and *S. haematobium* infection from 86 filtered urine samples collected in Ghana from low endemic area. Because, these low level infections will maintain the reservoir of infection and many infections will still persist following mass chemotherapy. It is important to detect and treat such infections. Our approach with PCR to amplify DNA from urine showed promising signs with much higher sensitivity (ranges from 99% - 100%) and specificity (100%) compared to KK and haematuria (sensitivity: 76% and 30%) for both schistosome species detection. High positive and negative predictive values (90% - 100%) were also indicative of robustness of PCR. The same pattern was observed when stratified for age group and sex specific analysis. In addition PCR detected 11 such individuals infected for both parasites who were considered not infected by either parasite. We have demonstrated that parasite specific DNA can be detected in urine when some specimens are apparently negative, as presence of DNA in the urine indicates a viable infection is still present. Our approach of disclosure of schistosome infection from filtered urine samples by PCR can be an effective means to detect low intensity infection and would enhance the effectiveness of surveillance and MDA control programs of schistosomiasis.

953

SCHISTOSOME ABC MULTIDRUG TRANSPORTERS: ROLES IN PARASITE PHYSIOLOGY, DRUG SUSCEPTIBILITY AND IMMUNOMODULATORY SIGNALING

Robert M. Greenberg, Beena John, Christopher A. Hunter, Ravi S. Kasinathan

University of Pennsylvania, Philadelphia, PA, United States

Members of the ATP-binding cassette (ABC) superfamily of proteins are efflux transporters that remove toxins and xenobiotics from cells. They likely play key physiological roles in excretion of wastes and metabolites, and they also transport a variety of signaling molecules, including those that have immunomodulatory activity. ABC transporters were originally characterized based on their role in mammalian multidrug resistance (MDR), and changes in structure or expression of these proteins are also associated with drug resistance in parasites, including helminths. Based on these properties, we postulate that these proteins can be considered attractive candidate targets for novel antischistosomal agents. We have previously shown that expression of two *Schistosoma mansoni* ABC transporters, P-glycoprotein (Pgp; SMDR2) and multidrug resistance associated protein (MRP1; SmMRP1) is altered in worms exposed to praziquantel (PZQ), the current drug of choice against schistosomiasis. Higher basal expression of these proteins is found in schistosomes with reduced PZQ susceptibility, including isolates exhibiting resistance to PZQ. PZQ inhibits SMDR2, and is also a likely substrate, and knockdown and pharmacological experiments indicate that SMDR2 and SmMRP1 play a role in schistosome egg production. In current experiments, we are using genetic and pharmacological approaches to assess whether disruption of parasite multidrug transporter expression or function enhances PZQ activity against adult and juvenile worms. We are also using similar approaches to assess the role these transporters presentation of parasite molecules that influence the host's immune response.

954

GENETIC BASIS OF DRUG RESISTANCE AND SPECIES-SPECIFIC DRUG ACTION IN SCHISTOSOME PARASITES

Claudia L. Valentim[1], Donato Cioli[2], Frederic Chevalier[3], Xiaohang Cao[1], Alex Taylor[1], Livia Pica-Mattoccia[2], Annalisa Basso[2], Alessandra Guidi[2], Isheng J. Tsai[4], Matthew Berriman[4], Claudia Carvalho-Queiroz[1], Marcio Almedia[3], Hector Aguilar[5], Doug E. Frantz[5], P. John Hart[1], Timothy J. Anderson[3], **Philip T. LoVerde**[1]

[1]University of Texas Health Science Center, San Antonio, TX, United States, [2]Institute of Cell Biology, CNR, Rome, Italy, [3]Texas Biomedical Research Institute, San Antonio, TX, United States, [4]Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, United Kingdom, [5]University of Texas at San Antonio, San Antonio, TX, United States

Oxamniquine kills *Schistosoma mansoni* but not *S. haematobium* or *S. japonicum*. High level resistance to oxamniquine evolved in the human blood fluke, *S. mansoni,* in Brazil in the 1970s and has been selected in laboratory populations. We exploited the genome sequence and genetic

map to identify the mutations underlying this trait, to determine the mode of drug action and the basis for species specific drug action. We staged a cross between parental parasites differing ~500-fold in drug response, determined drug sensitivity in clonally-derived F2s, and identified a single QTL (LOD=31) on chromosome 6. The causative gene, which encodes a sulfotransferase (SmSULT), was identified using RNAi knockdown and biochemical complementation assays, and we demonstrate independent origins of loss-of-function mutations in field-derived and laboratory-selected resistant parasites. By identifying the gene and specific mutations underlying drug resistance, we were able to confirm the mechanism of action. SmSULT sulfonates oxamniquine by catalyzing the transfer of a sulfuryl group (SO_3) from the active sulfate donor such as 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to oxamniquine to activate the drug, which binds to schistosome DNA, interfering with DNA synthesis and transcription, and killing the adult worms. In the laboratory-selected resistant parasite (HR) an amino acid deletion close to the active site interferes with drug binding, while in the field derived resistant isolate (MAP) a C→R mutation disrupts enzyme tertiary structure. These results demonstrate the utility of linkage mapping in a major human helminth parasite. Ongoing crystallography studies of protein-drug interactions demonstrate the structural relationship between the sulfate donor (PAPS), the sulfotransferase (SmSULT) and the drug (Oxamniquine), while phylogenetic studies revealed close homologues of the incriminated *S. mansoni* gene in *S. haematobium* and *S. japonicum*. These studies pave the way for rational design of second generation oxamniquine derivatives that can kill all human-infective schistosome species.

955

IMMUNE RESPONSES TO CHOLERA: LESSONS LEARNED FROM CHILDREN

Daniel T. Leung[1], Taher Uddin[2], Md. Murshid Alam[2], Md. Arif Rahman[2], Amena Aktar[2], Md. Arifuzzaman[2], Fahima Chowdhury[2], Ashraf I. Khan[2], Farhana Khanam[2], Taufiqur R. Bhuiyan[2], Jason B. Harris[1], Stephen B. Calderwood[1], Firdausi Qadri[2], Edward T. Ryan[1]

[1]Division of Infectious Diseases, Massachusetts General Hospital, Boston, MA, United States, [2]Centre for Vaccine Sciences, International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

Cholera is an acute dehydrating diarrheal disease caused by *Vibrio cholerae* O1 or O139 infection. In endemic areas, young children bear a high burden of disease; however, currently available oral cholera vaccines (OCV) give a lower efficacy and shorter duration of protection in this group than in older children and adults. The immunological reasons responsible for this are unclear. We have shown that in natural infections, young children achieve comparable antigen-specific antibody, gut-homing antibody secreting cell, and memory B cell (MBC) responses as adults. Conversely, children given OCV (Dukoral) are unable to develop detectable MBC responses against *V. cholerae* lipopolysaccharide (LPS), though significant increases in cholera toxin subunit B (CtxB)-specific MBC are seen. Notably, when compared to age-matched patients, child vaccinees have significantly lower, or absent, antibody and MBC responses to LPS and the O-specific polysaccharide, its major antigenic component. Furthermore, in these child vaccinees, increased CtxB-specific antibody avidity persists beyond one year, but avidity against LPS falls to baseline levels by one month after completion of vaccination. We also show that older child vaccinees mount significant CtxB-induced effector memory T cell (Tem) responses accompanied by prominent Th1 and Th2 cytokine secretion. Such Tem responses correlate with subsequent MBC levels up to 30 days, as well as increased antibody avidity up to one year. Younger child vaccinees, on the other hand, exhibit a lack of Tem response, and instead mount a prominent Treg response. Our findings suggest that young children given the OCV mount a different T cell response to the T-dependent antigen CtxB than older children, and most importantly, are unable to mount a durable memory immune response to LPS, the antigen believed to be most important in protection. These findings may explain the lower level of protection afforded to young children by vaccination.

956

ENVIRONMENTAL SURVEILLANCE FOR TOXIGENIC *VIBRIO CHOLERAE* IN SURFACE WATERS OF HAITI

Amy Kahler[1], Bradd Haley[2], Arlene Chen[2], Cheryl Tarr[1], Lee Katz[1], Maryann Turnsek[1], Rita Colwell[2], Anwar Huq[2], Vincent Hill[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]University of Maryland, College Park, MD, United States

The cholera epidemic that began in Haiti in 2010 has sickened more than 500,000 people. As outbreaks of cholera were not previously reported in Haiti, there is no information regarding the environmental presence of toxigenic *Vibrio cholerae* in Haitian surface waters. During each of 4 field visits (October 2011, March and August 2012, and January 2013), 19 river, canal, lake, and marine water sites were surveyed for the presence of toxigenic *V. cholerae*. Water samples were collected by 1-L grab sampling, 100-L dead-end ultrafiltration, and 100-L plankton net sampling. Samples were enriched overnight in alkaline peptone water (APW) and TCBS agar was used to isolate *V. cholerae* colonies. Real-time PCR assays targeting the *omp*W, *ctx*A, and *tcp*A genes were used to identify potential toxigenic *V. cholerae* isolates. APW enrichments were also screened directly for the presence of cholera toxin gene sequences (*ctx*A). Toxigenic *V. cholerae* was isolated from a river in the Artibonite Department in October 2011 and again from a different river in the same department in January 2013. Whole genome sequencing revealed that these isolates were a match to the outbreak strain. Cholera toxin (*ctx*A) was detected at 11 sites in October 2011 and 5 sites each in August 2012 and January 2013. Cholera toxin was not detected from any site in March 2012. Results of this survey demonstrate that toxigenic *V. cholerae* is present in surface waters in Haiti more than two years after the onset of the epidemic.

957

CHOLERA EPIDEMIC ASSOCIATED WITH UNSAFE DRINKING WATER AND STREET-VENDED WATER - EASTERN FREETOWN, SIERRA LEONE, 2012

Von Nguyen[1], Nandini Sreenivasan[1], Eugene Lam[1], David Kargbo[2], Foday Dafae[2], Amara Jambai[2], Wondimagegnehu Alemu[3], Tracy Ayers[1], Steven Stroika[1], Cheryl Bopp[1], Eric Mintz[1], Robert Quick[1], Joan Brunkard[1]

[1]Centers for Disease Control and Prevention, Atlanta, GA, United States, [2]Ministry of Health and Sanitation, Freetown, Sierra Leone, [3]World Health Organization, Sierra Leone Country Office, Freetown, Sierra Leone

Vibrio cholerae causes an estimated 3 million illnesses and 100,000 deaths annually. During 2012, Sierra Leone experienced a severe cholera epidemic with 22,252 reported cases and 292 deaths. In August 2012, CDC assisted the Ministry of Health and Sanitation in an outbreak investigation. The investigation team conducted a matched case-control study to assess risk factors for cholera. Cases were defined as acute watery diarrhea requiring IV hydration in persons ≥5 years old, presenting to a health facility from September 10-21. Controls were matched by age and neighborhood. Stool specimens from case-patients were analyzed by culture and polymerase chain reaction (PCR) for *V. cholerae*; isolates were subtyped by pulsed-field gel electrophoresis (PFGE). Conditional multivariate logistic regression was performed to investigate cholera risk factors. We enrolled 49 cases and 98 matched controls. Virtually all cases (96%) and controls (96%) obtained drinking water from improved water sources, such as boreholes and public taps. Consuming unsafe water (matched odds ratio [mOR]: 3.4; 95% confidence interval [CI]: 1.1, 11.0), street-vended water (mOR: 9.4; 95% CI: 2.0, 43.7) and crab (mOR: 3.3; 95% CI: 1.03, 10.6) were significant risk factors for cholera infection. Of 31 stool specimens from cases, 13 (42%) showed PCR evidence of toxigenic *V. cholerae*. Three specimens yielded isolates of *V. cholerae* O1, El Tor; all were resistant to co-trimoxazole and susceptible to doxycycline, ciprofloxacin, and tetracycline. PFGE analysis identified a pattern previously observed in seven countries. Testing of stored drinking water demonstrated evidence of chlorination in 28% of case and 38% of control households. Despite

near universal access to improved water sources, consuming unsafe water and street-vended water were risk factors for cholera infection. We recommended that prevention efforts focus on enhancing the microbiologic quality of improved water sources, promoting household drinking water chlorination, and improving street vendor water handling practices.

958

OBSERVATIONS FROM THE MASS VACCINATION PROGRAM WITH THE ORAL CHOLERA VACCINE, SHANCHOL, IN A HIGH RISK URBAN SETTING IN DHAKA, BANGLADESH 2011-2013

Amit Saha[1], Fahima Chowdhury[1], Ashraful Islam Khan[1], Iqbal Ansary Khan[1], Farzana Begum[1], Leanne Unicomb[1], Stephen P. Luby[2], Mohammed Ali[3], Alejandro Cravioto[3], John D. Clemens[1], **Firdausi Qadri**[1]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh, [2]Stanford University, Stanford, CA, United States, [3]International Vaccine Institute, Seoul, Republic of Korea

A large feasibility study on an oral cholera vaccine, Shanchol was conducted in a high risk urban population in Mirpur in Dhaka, Bangladesh. The aim was to determine to the feasibility of delivery and vaccination strategies utilizing the existing national immunization system. Bangladesh faces biannual peaks of cholera each year with 450,000 severe cases and at least a million infections. The need to utilize an affordable cholera vaccine as a public health intervention in a cholera prone country appeared extremely important in the national and regional context. The design of the feasibility study included vaccination together with a behavior change communication strategy in urban Mirpur, in six wards with a high cholera hospitalization rate. Following a geographic information system (GIS) approach, a census was carried out and the study was conducted using a cluster randomized design. Consent was obtained from eligible participants and bar coded identification cards were provided to individuals for census updates and to identify them during passive surveillance for cholera. Three arms of the program included a vaccine (n=80,000), a vaccine plus behavior change communication (n=80,000) as well as a non-intervention control arm (n=80,000). The vaccination program with Shanchol was conducted from the 17th of February to the 16th of April, 2011. Of the 171,110 target population (excluding those aged <1yr and pregnant woman), vaccination was carried out in about 141,882 individuals. About 87% of coverage was obtained for two doses of the vaccine. Thus, over 265,577 doses of Shanchol was delivered in less than two months using fixed site vaccination as well as mop up activities following the EPI procedure. Passive surveillance for detecting culture confirmed cholera cases were based on visits by participants to health facilities. The study has completed two years of surveillance in April 2013. Results of the effectiveness of the interventions and lessons learnt from the feasibility study so far will be presented.

959

COMPARATIVE PROTEOMIC ANALYSIS REVEALS ACTIVATION OF INNATE IMMUNE SIGNALING PATHWAYS AND THE INFLAMMASOME IN BANGLADESHI CHOLERA PATIENTS

Crystal N. Ellis[1], Leslie Mayo-Smith[1], Atiqur Rahman[2], Taher Uddin[2], Regina C. LaRocque[1], Edward T. Ryan[1], Stephen B. Calderwood[1], Firdausi Qadri[2], Jason B. Harris[1]

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

Cholera, caused by the bacterium *Vibrio cholerae*, is responsible for 3-5 million cases of diarrheal disease per year. Genome-wide studies of cholera patients in Bangladesh show that host genetic characteristics play an important role in susceptibility. These "omics" studies elucidate the poorly understood interaction between *V. cholerae* and the human host and provide insight for vaccine development. We collected duodenal biopsies from cholera patients in Bangladesh, 2 days post infection and again 30 days later when symptoms were absent. We analyzed tissue samples using label-free mass spectrometry (MS/MS) and MaxQuant, a protein quantification program, and compiled a list of 121 proteins that were differentially abundant ($p < 0.05$). Of those proteins, 34 were immunologically relevant including innate defense proteins previously identified in a study of acute cholera. We then used pathway analysis software to identify molecular interactions among proteins in our dataset and between other proteins in a reference database. Several proteins in our dataset were connected to innate immune pathways involved in TLR4-IFN signal transduction, NF-kB activation, and regulation of the NALP3 inflammasome. We confirmed differential abundance of two candidate proteins, WARS and S100A8, using immunohistochemistry. Involvement of WARS in *V. cholerae* infection and innate immunity has not previously been examined, and we speculate that WARS may activate certain pathways in a novel, cholera-specific manner. Recently, we have been developing an *in vitro* cell culture model of *V. cholerae* infection and we intend to use RNA interference in the context of this model to perform knockdown studies of several candidate proteins, including WARS and S100A8. Using this combined *in vivo* and *in vitro* approach, we can elucidate the role of these proteins in cholera pathogenesis and contribute to the understanding of human-*Vibrio* interaction at the gut mucosal surface.

960

THE RELATIONSHIP BETWEEN THE ANTIBIOTIC RESISTANCE AND VIRULENCE OF *ESCHERICHIA COLI*: A COMMUNITY-BASED STUDY IN RURAL ECUADOR

Lixin Zhang[1], Karen Levy[2], Gabriel Trueba[3], Joseph N. Eisenberg[1]

[1]University of Michigan, Ann Arbor, MI, United States, [2]Emory University, Atlanta, GA, United States, [3]Universidad San Francisco de Quito, Quito, Ecuador

Antimicrobial resistance is one of the most pressing issues in global health. Resistance can occur in any bacteria, but resistance in virulent bacteria (i.e. pathogens) is more threatening because of concerns about the consequences of an untreatable infection. Resistance may be linked to virulence as the result of differential antibiotic selection pressure and physical linkages between resistance genes and virulence genes. However, such relationships have rarely been evaluated with a community-based epidemiological study. In this case-control study of diarrheal diseases in northwestern Ecuador during 2009-2010, we systematically identified both pathogenic (n=86) and commensal (n=761) *Escherichia coli* isolates from 25 rural villages. Using antibiogram data for 12 antibiotics, *E. coli* genotypes, and resistance and virulence gene data, we assessed whether antibiotic resistance is linked with virulence in E .*coli* and explored factors that might contribute to such linkage. We observed that resistance to both individual and multiple antibiotics was higher in *E. coli* isolated from cases than from controls, and even higher in pathogenic versus commensal *E. coli*. Using a generalized estimating equation we found that pathogen status was more strongly related to antibiotic resistance than case status or antibiotic use for all antibiotics except quinolones. There was only a weak positive correlation between three out of four resistance genes and the virulence genes we tested. But pathogens and commensal *E. coli* differed significantly with regard to phylogroup distribution. These data suggest that the virulence and antibiotic resistance in *E. coli* are linked phenotypes, and the genetic background likely contributes to this linkage. Antimicrobial stewardship in the community setting is needed to address the concern that antimicrobial use may contribute to the persistence and spread of more virulent bacteria.

961

MAPPING THE EPIDEMIOLOGY OF YAWS IN THE SOLOMON ISLANDS

Michael Marks[1], Oliver Sokana[2], Ventis Vahi[2], Freda Pitaka[2], Cheng Y. Chen[3], Kai-Hua Chi[3], Alex Pavluck[4], Stephanie J. Migchelsen[1], Anisha Elisha[5], Zaixing Zhang[5], Kingsley Asiedu[6], Ronald Ballard[3], Allan Pillay[3], David C. Mabey[1], Anthony W. Solomon[1]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Solomon Islands Ministry of Health and Medical Services, Honiara, Solomon Islands, [3]Centers for Disease Control and Prevention, Atlanta, GA, United States, [4]Task Force for Global Health, Atlanta, GA, United States, [5]Western Pacific Regional Office, World Health Organization, Honiara, Solomon Islands, [6]World Health Organization, Geneva, Switzerland

Yaws is a re-emerging endemic treponemal infection, with the Pacific Islands a major focus of infection. The WHO is aiming to eradicate yaws by 2020 with a strategy that is heavily reliant on single-dose azithromycin treatment but there are scarce detailed epidemiological data to guide these efforts. Surveys of the Solomon Islands are ongoing to map the burden of disease. Incidence of reported clinical yaws in 2011 was 3,300 cases per 100,000 increasing to 5,382 cases per 100,000 in children aged ≤14 although cases are not routinely confirmed with serology. Data currently available for 2012 suggest a similar incidence in that year. There is considerable geographic variation within the country with the incidence varying between provinces from 1,353 to 8,418 cases per 100,000. These figures are likely to represent an underestimate of the true burden of disease. We have carried out population-based prevalence surveys in each of two provinces (Western and Choiseul) providing detailed data on both active and latent yaws infection. Over 1,000 children per province have been enrolled. A standardised questionnaire and examination were used to collect clinical data on both primary and secondary yaws. Whole blood was collected for serological testing (TPPA and RPR) on each subject, with a T. pertenue-specific PCR undertaken on individuals with suspected yaws ulcers and, a PCR to detect mutations associated with azithromycin resistance in *Treponema pallidum*. Independent review of skin-lesion photographs allows detection of additional skin disease phenotypes. We will present data on the prevalence of primary, secondary and latent yaws infection and the frequency of mutations conferring resistance to azithromycin. This work contributes significantly to our understanding of the epidemiology of both yaws and other skin diseases in the Solomon Islands and provides important data to inform yaws eradication efforts.

962

EVALUATION OF A MULTIVALENT VACCINE AGAINST LYMPHATIC FILARIASIS IN RHESUS MACAQUE MODEL

Gajalakshmi Dakshinamoorthy[1], Agneta von Gegerfelt[2], Hanne Andersen[2], Ramaswamy Kalyansundaram[1]

[1]University of Illinois Chicago, Rockford, IL, United States, [2]BIOQUAL, Inc., Rockville, MD, United States

Lymphatic filariasis affects 120 million people around the world and another 1.2 billion people are at risk. Chemotherapy with mass drug administration is substantially reducing the incidence of the infection. Nevertheless, an effective vaccine is needed to prevent the infection and eliminate the disease. Previously we reported that a multivalent fusion protein vaccine (rBmHAT) composed of small heat shock proteins 12.6 (HSP12.6), abundant larval transcript-2 (ALT-2) and large extracellular domain of tetrapsanin (TSP LEL) could confer >95% protection against *Brugia malayi* L3 challenge in mouse model. In this study we evaluated the immunogenicity and efficacy of the rBmHAT fusion protein vaccine in a Rhesus macaque model. A total of 10 rhesus monkeys were divided into two groups with five animals in each. Test animals received three intramuscular injections of 200µg of rBmHAT plus 200 µg of alum (IDRI) on days 0, 28 and 56. Control animals received only alum. Animals were bled after each dose and analyzed for vaccine induced antibody titers using an indirect ELISA. Our results show that all vaccinated monkeys developed significant titers of antigen-specific IgG antibodies (1:40,000) compared to controls. Antibody isotype analysis confirmed that the rBmHAT vaccination induced IgG1 production in response to all three antigens. Additionally, peripheral blood mononuclear cells from immunized animals proliferated significantly (S.I.-1.262±0.0738) in response to the vaccine antigen. Moreover, ELISPOT analysis confirmed that the antigen specific cells predominantly secreted IFN-γ. Finally, an *in vitro* antibody dependent cellular cytotoxicity (ADCC) assay showed that the antibodies in the sera of rBmHAT immunized animals participated in the killing of *Brugia malayi* L3 conferring 40% ±10.04 protection compared to controls. Taken together, this data is the first to demonstrate vaccine-induced protection against *B. malayi* in non human primates.

963

TARGETING IMMUNOSUPPRESSION AS A VACCINE STRATEGY AGAINST CHRONIC HELMINTH INFECTION

Simon A. Babayan

University of Glasgow, Glasgow, United Kingdom

The ability of helminths to ensure their survival and transmission rests upon their successful suppression of host immunity months- to decades-long. We predicted that by depleting immunomodulatory proteins which these parasites secrete, we could restore protective immune function to the host. Using *Litomosoides sigmodontis*, a filarial nematode that can complete its life cycle in laboratory mice, we reduced burdens of sexually mature adult parasites by 60-80% and their offspring by 90% using DNA vaccines expressing two genetically modified immunomodulatory proteins: abundant larval transcript LsALT-1 and cystatin LsCPI-2 of which we inactivated the immunosuppressive functions and targeted them specifically to dendritic cells (DC) using a sequence that encodes an anti-DEC205 single chain antibody. Unlike attenuated vaccines, which act immediately upon infection but not thereafter, the anti-immunomodulatory vaccines induced a gradual killing of *L. sigmodontis*. We have now identified several immune mechanisms *in vivo* and *in vitro* that these vaccines trigger, including IL4-independant increased production of IL12 and IL6 by DC, and increased proliferation of CD4+ T cells prior to challenge. During the chronic phase of infection, the same vaccine formulations, which also include host MIP1α- and IL4-expressing plasmids, induced stronger type-2 cellular and humoral immunity that correlated with worm killing. In conclusion, our results support our prediction that anti-immunodulatory vaccination would restore host immune function, and point to the importance of DC activation upon first encounter with helminths in building up protective immunity.

964

CHRONIC HELMINTH INFECTION PROTECTS MICE FROM TYPE III HYPERSENSITIVITY

Holly Evans, Edward Mitre

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

The mechanisms by which helminth infections protect against inflammatory diseases are not fully understood. In this study, we evaluated the effects chronic helminth infections have on type III hypersensitivity. Type III hypersensitivity, such as an Arthus reaction or serum sickness, is driven by antigen/antibody immune complex deposition and plays an important role in the pathogenesis of numerous diseases, including systemic lupus erythematosus. Mice were sensitized to ovalbumin (OVA) and then infected with *Litomosoides sigmodontis* for 10 weeks. Chronically infected animals exhibited significantly reduced ear swelling 12-24 hours after intradermal ear challenge with OVA ($p < 0.001$). Experiments using mice deficient in or depleted of CD4+ cells, mast cells, basophils, antibodies, or IgE demonstrated that this late phase inflammation was due to immune complex-mediated

type III hypersensitivity. Although total IgG and IgE levels were higher in sensitized + infected mice than sensitized mice, OVA-specific IgG1 ($p < 0.01$), IgG2a ($p < 0.05$), and IgE ($p < 0.001$) were all significantly reduced in mice that were chronically infected. After intradermal OVA challenge, immune complexes were detected by fluorescence microscopy in the tissues of both sensitized and sensitized + infected mice, with those in sensitized + infected mice larger and more focal than those in sensitized mice. Histological analysis of sensitized + infected mice showed reductions in edema, necrosis, and neutrophil and eosinophil numbers compared to sensitized animals. Although chronic helminth infection reduced circulating levels of C3 ($p < 0.01$), sensitized + infected mice exhibited the same kinetics of C3 decrease after OVA challenge as sensitized mice. In conclusion, these data demonstrate that a 10 week *L. sigmodontis* infection protects against type III hypersensitivity responses. Given the importance of immune complex reactions in the pathogenesis of numerous inflammatory diseases, these findings suggest a new mechanism by which helminth infections protect against inflammatory diseases. Future studies aim to further elucidate the immunological pathways mediating protection against type III hypersensitivity, and to evaluate whether worm-derived therapies can be useful in protection against diseases in which immune complex deposition is involved.

965

CHRONIC FILARIAL INFECTION IMPROVES *ESCHERICHIA COLI* INDUCED SEPSIS IN A TLR2 DEPENDENT MANNER

Fabian Gondorf, Achim Hoerauf, **Marc P. Hübner**

Institute for Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Bonn, Germany

Helminths modulate the immune system of their hosts and induce a regulatory, anti-inflammatory milieu that enables long-term parasite survival within the host, but may also benefit the host. Thus, helminth infections have been shown to improve allergies and autoimmune diseases. In the current study we investigated whether chronic infection with the filarial nematode *Litomosoides sigmodontis* (L.s.) improves the outcome of an acute systemic inflammation caused by i.p. *Escherichia coli* injection in BALB/c mice. Chronic L.s. infection significantly improved *E. coli* induced hypothermia, bacterial clearance and sepsis survival compared to *E. coli*-only injected controls. The L.s. mediated protective effect correlated with significantly increased levels of anti-inflammatory TGFβ and reduced concentrations of pro-inflammatory cytokines after *E. coli* challenge. Depletion of peritoneal macrophages prevented the filaria mediated protection against *E. coli* challenge. Improved bacterial clearance in L.s. infected animals was not due to an increased phagocytic capacity of macrophages, but correlated with a reduced loss of peritoneal macrophages and an alternatively activated phenotype after *E. coli* challenge. However, the L.s. mediated protective effect was still given in IL-4 and IL-4R/IL-5 deficient animals, suggesting that alternatively activated macrophages are not required. Endosymbiotic *Wolbachia* bacteria, that are present in most human pathogenic filaria and L.s., signal via TLR2 and may induce cross-tolerance to TLR4 stimuli. Accordingly, *in vitro* experiments revealed that pretreatment of macrophages with crude L.s. antigen reduced LPS induced activation and lack of TLR2 signaling in L.s. infected mice prevented the protection against *E. coli* challenge. Our study suggests that chronic L.s. infection can have a beneficial effect on acute bacterial infections. Current experiments are ongoing to test whether this is due to exposure to *Wolbachia* bacteria in chronically L.s. infected mice that prevents *E. coli* induced excessive macrophage activation by a TLR cross-tolerance mechanism.

966

IDENTIFICATION OF HUMAN TH9 CELLS: ANTIGEN-SPECIFIC, IL-4- AND TGFβ-DEPENDENT EXPANSION OF A DISCRETE NON TH2 CD4+ T CELL SUBSET AND THEIR ROLE IN A CHRONIC FILARIAL INFECTION

Anuradha Rajamanickam[1], Parakkal Jovvian George[2], Luke E. Hanna[3], Vedachalam Chandrasekaran[3], Paul Kumaran[3], Thomas B. Nutman[4], Subash Babu[2]

[1]NIH-ICER-NIRT, Chennai, India, [2]National Institutes of Health - International Center for Excellence in Research, Chennai, India, [3]National Institute for Research in Tuberculosis, Chennai, India, [4]Laboratory of Parasitic Diseases, National Institutes of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

Th9 cells are a subset of $CD4^+$ T cells producing IL-9 and IL-10 and shown to be important in allergy, autoimmunity and antitumor responses. However, their role in human infectious diseases has not been explored in detail. Lymphatic filariasis can be associated with the development of serious pathology in the form of lymphedema, hydrocele and elephantiasis in a subset of infected patients. We postulated that this infection would provide an ideal milieu to examine the role of Th9 cells. We identified a population of IL-9 and IL-10 co-expressing $CD4^+$ T cells (lacking IL-4 expression) in normal individuals that respond to antigenic and mitogenic stimulation but are distinct from $IL\text{-}9^+$ Th2 cells. We also demonstrate that these Th9 cells exhibit antigen -specific expansion in filarial infected individuals. Comparison of Th9 responses reveals that individuals with lymphedema and elephantiasis associated with *Wuchereria bancrofti* infection exhibit significantly ($p<0.0001$) expanded frequencies of filarial antigen induced Th9 cells but not of $IL9^+$Th2 cells in comparison to filarial-infected individuals without associated disease (subclinical infection). Moreover, the per cell production of IL-9 ($p<0.0001$) is significantly higher in Th9 cells compared to $IL9^+$Th2 cells, indicating that the Th9 cells are the predominant $CD4^+$ T cell subset producing IL-9 in the context of human infection. The expansion of $IL\text{-}9^+$ $CD4^+$ T cells was also reflected in elevated antigen stimulated IL-9 ($p<0.0001$) cytokine levels in whole blood culture supernatants. Finally, the baseline and antigen driven frequencies of Th9 cells correlated positively with the severity of lymphedema (and presumed inflammation) in filarial diseased individuals. This antigen driven expansion of Th9 cells was dependent on IL-4 and TGFβ as blockade of these two cytokines resulted in a significantly decreased expansion of Th9 cells in short term cultures. We have therefore identified an important human $CD4^+$ T cell subpopulation co-expressing IL-9 and IL-10 but not IL-4, whose expansion is associated with disease in chronic lymphatic filariasis and could potentially play an important role in the pathogenesis of many other inflammatory disorders.

967

THE EXPANDED REGULATORY T CELL POPULATION IN CHRONIC FILARIAL INFECTION IS HIGHLY HETEROGENEOUS, ACTIVATED AND SUPPRESSES CYTOKINE PRODUCTION DENDRITIC CELLS

Simon Metenou[1], Yaya I. Coulibaly[2], Daniel Sturdevant[3], Housseini Dolo[2], Abdallah A. Diallo[2], Lamine Soumaoro[2], Michel E. Coulibaly[2], Kishore Kanakabandi[3], Stephen F. Porcella[3], Amy D. Klion[1], Thomas B. Nutman[1]

[1]National Institutes of Health, Bethesda, MD, United States, [2]Pharmacy and Dentistry, University of Bamako, Bamako, Mali, [3]Rocky Mountain Laboratories, National Institutes of Health, Hamilton, MT, United States

Natural regulatory T cells (nTregs) are known to increase during chronic infection or at the site tumor, though the exact mechanisms that contribute to their accumulation and mode of action remain unclear. We used flow cytometry and microarray analyses to delineate the phenotype of nTregs and their role in modulating T cell and antigen presenting cell (APC) function in the setting of chronic infection. Using cells from 18 filaria-infected (Fil+) and 19 filaria-uninfected (Fil-) subjects, we found that the frequencies of nTreg expressing CTLA-4, GITR, LAG-3, and IL-10 were significantly higher in Fil+ compared with Fil- subjects. Microarray analysis revealed that, compared with those from Fil-, nTreg populations in Fil+ subjects were more heterogeneous and had higher expression of IL-10, CCL-4, IL-29 and CTLA-4, molecules that have been implicated in immune suppression. Moreover, the nTregs from Fil+ subjects had markedly upregulated activation-induced apoptotic genes with concomitant down regulation of cell survival genes. To determine if nTregs directly modulate APC function, we used an *in vitro* co-culture system with purified APCs and nTregs from Fil+ and Fil- subjects. In this system, in response to *Brugia malayi* antigen, APCs from Fil+ produced higher levels of IL-6 and TNF-α ($p = 0.01$ and $p = 0.04$, respectively). When APCs and nTregs were co-cultured together, in response to BMA stimulation APCs-nTregs co-culture from Fil+ only produced higher levels of IL-10 compared with APCs. Antibody blockade of nTregs surface makers (PD1, GITR, CTLA-4 and LAG-3) in nTregs-APCs co-cultures induced higher levels of IL-10 and TGF-β cells from Fil+ subjects only. Taken together, our results suggest that in filarial infection, the expanded nTreg populations are heterogeneous, short-lived, activated and express regulatory molecules that modulate cytokine production by APCs.

968

FAILURE OF HUMAN LANGERHANS CELLS TO INITIATE AN INFLAMMATORY RESPONSE FOLLOWING EXPOSURE TO FILARIAL INFECTIVE LARVAE (L3) SUGGESTS A MECHANISM FOR IMMUNE EVASION

Renee McDonald-Fleming, Rachel N. Cotton, Soumya Chatterjee, Thomas B. Nutman, Roshanak Tolouei Semnani

National Institutes of Health, Bethesda, MD, United States

Epidermal Langerhans cells (LC) and keratinocytes (KC) most likely are the first line of defense following the interaction with the infective third larval stage (L3) of *Brugia malayi*. Previous studies from our laboratory indicate that 48 hours exposure to L3 failed to activate human LC but did, to a small degree, functionally suppress these cells. To assess whether longer exposure of epidermal LC to the parasite is required to induce an innate immune response, we used an *ex vivo* human skin blister model as well as *in vitro* generated LC. Our data indicate that longer L3 exposure (3-5 days) to human epidermal skin blisters (but not to the *in vitro* generated LC) downregulated the mRNA expression of thymic stromal lymphopoietin (TSLP) and significantly inhibited the production of TNFα ($p=0.02$) but not inflammasome-associated cytokines IL-18 and IL-1RA. The difference between the blisters and *in vitro* generated LC in TNF-α and TSLP downregulation by L3 may suggest for the role of KC in response to the parasite. Because toll like receptors (TLR) play an important role in pathogen recognition, we next determined the effect of L3 on LC TLR function. *In vitro* generated LC were exposed to live L3 (or other stimuli) and then assessed for responses to ligands for TLR-2, -3, -4, and -6) using Luminex™. Our data suggests that exposure to L3 did not alter the production of any of the cytokines measured including IL-10, IL-12p40, IL-1α, IL-1β, TNFα, and IL-6. Taken together, these data suggest that the muted response to L3 by LC even at longer exposure may be a mechanism by which the parasite escapes the consequences of the innate barrier associated response. Moreover, the presence of KC may be important in mediating the LC response to the parasite.

969

DIHYDROARTEMISININ-PIPERAQUINE VS. ARTEMETHER-LUMEFANTRINE FOR FIRST-LINE TREATMENT OF UNCOMPLICATED MALARIA IN AFRICAN CHILDREN: A COST-EFFECTIVENESS ANALYSIS

Yesim Tozan[1], Johannes Pfeil[2], Steffen Borrmann[2]

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

Recent trials in Africa showed that dihydroartemisinin-piperaquine (DHAPQ), a newer fixed-dose artemisinin-based combination therapy (ACT), was as efficacious and safe as artemether-lumefantrine (AL) for treatment of young children with uncomplicated malaria across different endemicity settings. Longitudinal follow-up of patients also confirmed that DHAPQ had a longer post-treatment prophylactic effect than AL, reducing the risk of recrudescence and reinfection and hence conferring additional health benefits to patients. We estimated the threshold costs of DHAPQ per course treatment for which DHAPQ would be a cost-effective alternative to AL, given the post-treatment prophylactic effects of these drugs. Decision analysis was performed using a Markov model that simulated incidence of uncomplicated and severe malaria, survival, disability adjusted life years (DALYs) and costs in a hypothetical cohort of children receiving DHAPQ or AL for treatment of uncomplicated malaria. We calculated weekly hazard rates for recurrent malaria after treatment with DHAPQ and AL, using the published data on the proportion of patients whose treatment was failure free by day of follow up from a multi-center randomized control trial in Africa. At a mean cost of $0.36 per treatment course for both drugs, first-line treatment with DHAPQ was the dominant treatment strategy over AL with an improvement of 0.05 DALYs averted per child (95% CI 0.01--0.12) and a cost saving of $2.06 per child (95% CI 0.48--5.09). First-line treatment with DHAPQ remained the dominant treatment strategy (less costly and more effective) for any cost per course treatment below $1.27. Between $1.27 and $1.78, first-line treatment with DHAPQ was a "highly attractive" intervention compared to AL with an incremental cost-effectiveness ratio less than "$25 per DALY averted" under World Bank guidance. This study demonstrates the superiority of DHAPQ over AL for the treatment of uncomplicated *Plasmodium falciparum* malaria in young children from a clinical and economic perspective. Substituting DHAPQ with AL as first-line antimalarial treatment in highly endemic areas of Africa merits serious consideration by health policy makers.

970

EFFICACY AND ACCEPTABILITY OF ARTEMETHER-LUMEFANTRINE VERSUS DIHYDROARTEMISININ-PIPERAQUINE IN KENYAN CHILDREN WITH UNCOMPLICATED *FALCIPARUM* MALARIA

Bernhards Ogutu[1], Kevin Omondi Onyango[2], John Michael Ongecha[3], Elizabeth Juma[3], Godfrey Allan Otieno[1], Charles Obonyo[3], Lucas Otieno[1], Fredrick Eyase[4], Jacob D. Johnson[4], Douglas Jay Perkins[5], Willis Akhwale[6]

[1]Walter Reed Project-Centre for Clinical Research, Kenya Medical Research Institute, Nairobi, Kenya, [2]Walter Reed Project-Centre for Clinical Research, Kenya Medical Research Institute, Kisumu, Kenya, [3]Walter Reed Project-Centre for Global Health Research, Kisumu, Kenya, [4]Walter Reed Project, Kisumu, Kenya, [5]University of New Mexico, Albuquerque, NM, United States, [6]Department of Disease Prevention and Control, Ministry of Public Health and Sanitation, Nairobi, Kenya

Artemether-lumefantrine (AL) and dihydroartemisinin-piperaquine (DP) have been introduced as first and second-line treatment, respectively, for uncomplicated *falciparum* malaria in Kenya. This open-label, randomized, comparative study in Western Kenya compared corrected Acceptable Clinical and Parasitological Responses (ACPR) in children aged 6 to 59 months, treated with AL-dispersible (AL_d) and DP-pediatric (DP_p). Adherence and acceptability of both drugs were also assessed. Children were hospitalized for 3-days to receive either AL_d (n=227) or DP_p (n=227) and followed up on Days 7, 14, 28 and 42. Adherence and acceptability were assessed by caregiver questionnaire, including general questions with respect to preferred pediatric formulations. No significant differences were observed for corrected ACPR rates on Days 14, 28 and 42 for AL_d (100%, 97.8%, and 96.8%, respectively) and DP_p (100%, 99.1%, and 98.7%, respectively; p>0.05). Similar results were seen for uncorrected ACPR rates. Overall incidence of adverse events was 65.5% (156/238) and 67.5% (156/231) in AL_d and DP_p arms. The adherence to treatment regimen was higher for children treated with AL_d (93.6%) compared to DP_p (85.6%). 82% of the caregivers considered AL_d 'simple' or 'very simple' to use compared with 67% in DP_p arm (p=0.007). The taste of AL_d was 'liked' or 'liked very much' by 72% of respondents, compared with 56% of respondents for DP_p (p=0.001). The majority in both groups took the drug with a meal (AL_d=94.4%; DP_p=89.4%), and preferred water to dissolve the tablets (AL_d=94.4%; DP_p=89.4%). In general, caregivers preferred the dispersible tablet formulation (drug given as tablet dissolved in a small volume of water/milk) as compared to a syrup formulation (AL_d=76.8% vs. 16.8%; DP_p=62.3% vs. 29.5%). Both, AL_d and DP_p are efficacious treatments for uncomplicated *falciparum* malaria in Kenyan children. Acceptability of AL_d regimen was assessed as being significantly better than DP_p in the caregiver survey.

971

TREATMENT EFFICACY OF ARTESUNATE-AMODIAQUINE TREATMENT REGIMENS FOR UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA: COMPARISON OF FIXED VERSUS CO-BLISTER FORMULATIONS

Christian Nsanzabana, On behalf of WWARN ASAQ Dosing Impact Study Group

WorldWide Antimalarial Resistance Network, Oxford, United Kingdom

Artesunate-amodiaquine (AS-AQ) is the first line antimalarial treatment in 25 countries and it is available in non-fixed dose formulations (nFDC), either loose or co-blister. A fixed dose combination (FDC) of AS-AQ was introduced in 2007 to optimize the AS-AQ ratio and improve adherence. Current dosing for the FDC is according to 3 age ranges with 3 dosing strengths available. To assess the spectrum of total weight adjusted dosages (mg/kg) of AQ administered and to compare the effect of fixed and non-fixed AS-AQ combinations on treatment efficacy, individual patient data were shared with WWARN and collated using standardised methodology. Factors associated with Polymerase chain reaction (PCR)-confirmed recrudescences were assessed using a Cox's regression model with shared frailty on study sites. Data from 6,140 patients (26 efficacy studies, between 2002 and 2011) with uncomplicated *P. falciparum* malaria were included in the analysis [6,043 from Africa, 97 from Asia]. 22 studies (n=5,563) had a follow up of 28 days and 4 studies followed patients for 42 days or longer (n=577). 167 (2.7%) PCR-confirmed recrudescent parasitaemia were observed. Patients treated with the FDC received a higher median (IQR) mg/kg dose of AQ [33.8 mg/kg (27.5-40.5)] compared to those receiving nFDC [27.0 mg/kg (25.0-35.0)] (p<0.001). In a multivariate model, independent risk factors associated with recrudescence were: young age (1- <5 years old) (AHR=5.41 [95% CI: 1.58-18.47], p=0.007 compared to aged≥12 years) and log of the baseline parasitaemia (AHR=1.26 [95% CI: 1.10-1.43, p<0.001). The mg/kg dose of AQ was not a significant risk factor. After adjusting for confounding factors, the use of non-fixed dose formulations was associated with 2.8 fold increased risk of treatment failure [AHR: 2.85, 95% CI: 1.58-5.10, p<0.001]. The fixed dose formulation provides better efficacy than loose combinations, and this likely due to improved dosing. In addition, it is expected that FDC would improve adherence, effectiveness and eliminate the risk of using either drug as monotherapy, one of the drivers of resistance.

972

CARRIAGE OF DRUG RESISTANT *PLASMODIUM FALCIPARUM* IN MALARIA/HIV CO-INFECTED PATIENTS AND IMPROVEMENT OF CD4+ COUNTS AFTER ARTEMETHER-LUMEFANTRINE TREATMENT IN PORT HARCOURT, NIGERIA

Ifeyinwa N. Chijioke-Nwauche[1], Chijioke A. Nwauche[2], Kalada J. Atemie[3], Colin J. Sutherland[4]

[1]Department of Immunology and Infection, London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Centre for Malaria Research and Phytomedicine, University of Port Harcourt, Port Harcourt, Nigeria, [3]Department of Haematology, Blood Transfusion and Immunology, University of Port Harcourt, Port Harcourt, Nigeria, [4]Department of Immunology and Infection, London School of Hygiene & Tropical Medicine, London, United Kingdom

The occurrence of malaria in HIV infected persons is a major challenge to public health. HIV increases the risk of malaria infection and clinical malaria in adults in areas of stable transmission especially in those with advanced immunosuppression. HIV also increases frequency of drug intake and thus drug pressure. This may contribute to the emergence and spread of drug resistance. Higher parasite burdens as seen in HIV subjects may also increase the likelihood of carrying drug resistant parasites. Studies of molecular markers of antimalarial drug resistance have usually not included HIV subjects. The study was carried out in Port Harcourt, Nigeria, rich in the nation's oil resources and with high malaria transmission rates. Due to the oil and gas activities in the area, there is a large number of migrant workers with commercial sex workers following the camp resulting in high prevalence of HIV infection. Asymptomatic *Plasmodium falciparum* carriers were identified by microscopy among adult attendees at the HIV clinics and HIV-negative people from University of Port Harcourt Teaching Hospital community. Participants were treated with artemether-lumefantrine (AL)and followed up; blood samples were collected on D0, D3 and D28. Assaying for molecular markers was carried out and CD4+ T-cells counted. Paired peripheral blood CD4 cell counts at both day 0 and day 28 were available for 38 HIV volunteers. Over 28 days following, we observed a mean increase of 107.3 cells per µl (95% CI 53.8-160.8; P=0.0002). Using the PCR data as a more reliable test for parasite carriage, we found that 24 of these volunteers had confirmed parasites and 19 of these had cleared the parasites by D28 after treatment with AL. Genotype data at the pfmdr1, pfcrt and antifolate resistance marker *loci* pfdhfr and pfdhps will be presented.

973

PRIMAQUINE FAILURE IN *PLASMODIUM VIVAX* THERAPY IS CAUSED BY A DEFICIENCY IN CYTOCHROME P450 2D6

Jason W. Bennett[1], Brandon S. Pybus[1], Anjali Yadava[1], Donna Tosh[1], Jason C. Sousa[1], William F. McCarthy[2], Gregory Deye[1], Victor Melendez[1], Christian F. Ockenhouse[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]U.S. Army Medical Research and Materiel Command, Frederick, MD, United States

Primaquine (PQ) is the only FDA-approved medication to treat the hypnozoites of *Plasmodium vivax*, but relapses due to drug failure occur. Human cytochrome P450 isoenzyme 2D6 (CYP2D6) metabolizes PQ and may play a critical role in production of active PQ metabolites in the liver. We sought to identify an association between CYP2D6 activity and PQ drug failure. 33 subjects were challenged with *P. vivax* sporozoites by the bites of infected mosquitoes. Beginning on the day of *parasitemia*, all subjects were treated for with a combination of chloroquine (1500 mg base over 48 hours) and PQ (30 mg base by mouth daily for 14 days) under directly observed therapy. Subjects were monitored for malaria relapse for >12 months. Upon relapse, chloroquine dosing was repeated; however, PQ dosing was weight-based to acheive a total dose of 6 mg/kg. CYP2D6 phenotypes were ascertained in 25 subjects. The pharmacokinetics of PQ metabolism was determined using plasma from these subjects. All subjects became parasitemic by day 13 post challenge and rapidly cleared *parasitemia* upon initiation of therapy. Two subjects (6%) experienced malaria relapses. CYP2D6 phenotyping revealed 21 (84%) extensive metabolizers (EM), 3 (12%) intermediate metabolizers (IM), and 1 (4%) poor metabolizer (PM). There were 0 relapses in the EM group, 2 relapses in one IM subject, and 3 relapses in the PM subject. Rapid clearance of blood stage parasites was documented by PCR following the initiation of re-treatment for relapses. There was a significant association between relapse and CYP2D6 phenotypes associated with low isoenzyme activity. Pharmacokinetic analysis demonstrated markedly higher plasma concentrations of parent PQ in relapse subjects consistent with decreased metabolism by CYP2D6. CYP2D6 activity appears to be critical in metabolizing PQ to its active metabolite. In this small population of subjects, deficiency of CYP2D6 isoenzyme activity was associated with failure of PQ to prevent relapse of *P. vivax*. Larger study populations are necessary to further elucidate the relationship between CYP2D6 activity, geographic regional dosing requirements, and clinical failure of PQ for radical cure of hypnozoites.

974

QUININE TREATMENT IN PREGNANT WOMEN WITH MALARIA-HIV CO-INFECTION: PILOT OBSERVATIONAL STUDY

Kassoum Kayentao[1], Etienne A. Guirou[1], Ogobara K. Doumbo[1], Meera Venkatesan[2], Teresa L. Parsons[3], Craig W. Hendrix[3], **Myaing M. Nyunt**[4]

[1]Malaria Research and Training Center, University of Bamako, Bamako, Mali, [2]University of Maryland, Baltimore, MD, United States, [3]Johns Hopkins Bloomberg School of Medicine, Baltimore, MD, United States, [4]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Pregnant women bear the greatest burden of malaria-HIV co-infection. Quinine continues to be important in the treatment of uncomplicated malaria in early pregnancy and severe malaria. Previous studies suggest that HIV infection and interaction between antimalarial and antiretroviral drugs may negatively influence antimalarial pharmacokinetics and treatment outcomes. We conducted a pilot clinical study to assess quinine pharmacokinetics in Malian pregnant women co-infected with acute *falciparum* malaria and HIV. All ten participants had PCR-corrected 28-day adequate clinical and parasitologic responses. Adverse events were frequent. One of the ten women who reported taking nevirapine-based antiretroviral therapy showed no measurable concentrations of nevirapine in her plasma. Plasma concentration of both total and free (unbound) quinine were lower, and the major active metabolite 3-hydroxyquinine higher, in pregnant women who had a stable plasma concentration of nevirapine than in the participant who did not. Quinine trough concentrations were below the recommended therapeutic range of 5-15 mg/L in 50% of the women who provided plasma samples. The study findings suggest that chronic administration of nevirapine-based antiretroviral therapy may negatively influence quinine pharmacokinetics, and co-administration of these drugs may also increase the risk of drug toxicity. Further research is warranted to understand the impact of HIV as a chronic disease and its long term antiretroviral therapy on the treatment of acute malaria.

975

INTERMITTENT PARASITE CLEARANCE IN SCHOOLCHILDREN: IMPACT ON COGNITION IN AN AREA OF HIGHLY SEASONAL TRANSMISSION

Sian E. Clarke[1], Saba Rouhani[1], Seybou Diarra[2], Modibo Bamadio[2], Rebecca Jones[1], Diahara Traore[3], Matthew C. Jukes[4], Josselin Thuillez[5], Moussa Sacko[6], Simon Brooker[1], Sueng Lee[7], Natalie Roschnik[7]

[1]London School of Hygiene & Tropical Medicine, London, United Kingdom, [2]Save the Children, Bamako, Mali, [3]Programme National de Lutte contre le Paludisme, Ministry of Health, Bamako, Mali, [4]Room to Read, London, United Kingdom, [5]Centre d'économie de la Sorbonne, Paris, France, [6]Institut National de Recherche en Santé Publique, Ministry of Health, Bamako, Mali, [7]Save the Children, Washington, DC, United States

Malaria control has usually focused on those at most risk of malaria-related mortality (pregnant women and children under five). Yet recent studies show that older school-age children can also benefit from malaria control, with potential gains for both health and education. Mali introduced universal coverage of nets with community-wide distributions starting in 2011. Whilst successful in achieving high levels of coverage, 80% of schoolchildren remained infected at the end of the malaria transmission season in Nov 2011. This calls for additional control measures in this age group. One potential supplementary strategy is intermittent parasite clearance in schools (in which a treatment dose is given irrespective of infection status) with the aim to improve educational performance by reducing malaria-related anaemia and improving cognitive function among schoolchildren. This approach is particularly suited to areas of seasonal transmission where a single annual treatment can be given at the end of the transmission season. A cluster-randomized controlled trial was conducted in 80 primary schools in Sikasso, Mali where the majority of schoolchildren already slept under insecticide-treated nets. Children in intervention schools received a single treatment dose at the end of Nov 2011; administered in school by teachers over three consecutive days. Parasite clearance was associated with dramatic reductions in malaria parasitaemia and gametocyte carriage at follow-up in Feb 2012 in intervention compared to control schools. This effect was sustained until May 2012, the beginning of the next transmission season. Malaria parasite clearance was also associated with a significant decrease in anaemia (OR=0.56, 95% CI 0.39 to 0.78, p=0.001), and increase in sustained attention (p<0.001). Findings from the full battery of cognitive and educational tests will be presented, and discussed in relation to the growing body of evidence on the impact of asymptomatic malaria infection on cognitive performance in schoolchildren, and control strategies in areas of highly seasonal transmission.

976

PLASMODIUM FALCIPARUM PFS47 GENE MEDIATES EVASION OF THE MOSQUITO IMMUNE SYSTEM

Alvaro Molina-Cruz[1], Lindsey S. Garver[1], Amy Alabaster[1], Lois Bangiolo[1], Ashley Haile[1], Jared Winikor[1], Corrie Ortega[1], Ben C. van Schaijk[2], Robert W. Sauerwein[2], Emma Taylor-Salmon[1], Carolina Barillas-Mury[1]

[1]National Institutes of Health, Bethesda, MD, United States, [2]Radboud University, Nijmegen, Netherlands

Some *Plasmodium falciparum* lines are able to infect the refractory *Anopheles gambiae* L3-5 strain by evading its complement-like immune system. A Quantitative Trait *Loci* (QTL) mapping was carried out to identify the *P. falciparum* gene(s) that allow evasion of the *A. gambiae* immune system. The gene mapping was done in a cross between *P. falciparum* GB4 that successfully infects the A.*gambiae* L3-5 strain, and the 7G8 line which is eliminated in *A.gambiae* L3-5 by melanotic encapsulation. QTL analysis identified one main significant locus in chromosome 13 that is associated with the phenotype; this locus was confirmed independently by linkage group selection in individual oocysts. Candidate genes were selected for detailed genetic analysis based on gene expression differences and sequence polymorphisms between the parental lines. Knocking out Pfs47 in *P. falciparum* NF54, a line that infects *A. gambiae* L3-5, results in a line that is melanized in *A. gambiae* L3-5. This melanization is dependent on the mosquito complement-like system and can be rescued by genetic complementation with the wild type gene. The Pfs47 protein, a member of the 6-cys protein family, was found to be present in the surface of ookinetes. Taken together, all the evidence indicates that Pfs47 mediates *P. falciparum* evasion of the *A. gambiae* immune system. Pfs47 may be important to understand adaptation of the parasite to different Anopheline mosquitos around the world, and could be a target for transmission blocking strategies.

977

MULTIPLE MECHANISMS OF LATE-PHASE IMMUNITY LIMIT *PLASMODIUM* DEVELOPMENT IN THE MOSQUITO *ANOPHELES GAMBIAE*

Ryan C. Smith[1], Carolina Barillas-Mury[2], Marcelo Jacobs-Lorena[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

Mosquitoes of the genus *Anopheles* serve as the obligate vectors of the malaria parasite *Plasmodium*. During its development within the mosquito host, several factors and developmental bottlenecks limit parasite success, including two distinct phases of the mosquito innate immune response. Emerging evidence suggests that parasite numbers are largely influenced by an "early-phase" that targets ookinetes as they reach the basal lamina of the mosquito midgut through the exposure to complement-like components of the hemolymph and a "late-phase" response that limits oocyst survival through the production of nitric oxide mediated by the STAT pathway. Recently, we have identified a novel LITAF-like transcription factor (LL3) in *Anopheles gambiae* that is an important component of the mosquito response to *Plasmodium*. Following LL3-silencing, oocyst numbers are significantly increased and evidence suggests that LL3 is involved in the "late-phase" response by limiting oocyst survival. Experiments to determine the relationship between the "late-phase" response for LL3 and those previously described for the STAT pathway imply that the LL3 and STAT phenotypes occur through independent, yet closely related mechanisms involving hemocyte function. Current experiments aim to further dissect LL3 function to address a critical gap in our knowledge of the mosquito late-phase immune response and the mechanisms that limit oocyst survival in its mosquito host.

978

CHARACTERIZATION OF THE TARGET OF IVERMECTIN, THE GLUTAMATE-GATED CHLORIDE CHANNEL, IN *ANOPHELES GAMBIAE* AND AS A TARGET OF A MOSQUITOCIDAL VACCINE

Jacob I. Meyers, Benjamin J. Krajacich, Meg Gray, Lucas B. Johnson, Christopher D. Snow, Kathryn M. Partin, Brian D. Foy

Colorado State University, Fort Collins, CO, United States

The use of insecticide-treated nets and indoor residual insecticides targeting adult mosquito vectors is a key element in malaria control programs. However, the spread of insecticide-resistant mosquitoes has countered these efforts to control vector populations. Recently, the use of ivermectin mass drug administration has been shown to kill *Anopheles gambiae* mosquitoes and disrupt malaria transmission in the field. We cloned the molecular target of ivermectin from *An. gambiae*, the glutamate-gated chloride channel (AgGluCl), and characterized its protein expression and activity to glutamate and ivermectin. Cloning revealed four AgGluCl splice isoforms with heterogeneity between isoforms found in the N-terminal extracellular domain and the intracellular loop region. AgGluCl expression was observed throughout the mosquito nervous systems including neuropil associated with multiple sensory and motor systems. Activity was measured using two-electrode voltage clamp on *Xenopus laevis* oocytes expressing AgGluCl to glutamate and ivermectin. Ivermectin was shown to induce non-persistent AgGluCl activity and does not potentiate glutamate responses, which is unique among GluCls. An alternative strategy to chemical insecticides for parasite transmission control is the development of mosquitocidal and transmission-blocking vaccines. By feeding *An. gambiae* blood meals containing polyclonal IgG targeting AgGluCl, we were able to kill the majority of blood fed mosquitoes (LC_{50}: 2.82mg/mL). We show that blood meals containing AgGluCl IgG block AgGluCl activity, either through AgGluCl antagonism or internalization of AgGluCl from the membrane surface.

979

GENETIC MANIPULATION OF *ANOPHELES STEPHENSI* IMMUNITY TO INCREASE *PLASMODIUM FALCIPARUM* SALIVARY GLAND SPOROZOITE INFECTION LEVELS

Adam Richman[1], Peter Billingsley[1], Abraham Eappen[1], Tao Li[1], Sumana Chakravarty[1], B. Kim Lee Sim[1], Kasim George[2], Hanfu Xu[2], David A. O'Brochta[2], Stephen L. Hoffman[1]

[1]Sanaria Inc., Rockville, MD, United States, [2]Department of Entomology, Institute for Bioscience and Biotechnology Research, Rockville, MD, United States

Sanaria's technology platform generates live, aseptic, purified, cryopreserved *Plasmodium falciparum* sporozoites (PfSPZ) that can be administered as a highly protective malaria vaccine. PfSPZ are produced using *Anopheles stephensi* mosquitoes as bioreactors. The capacity of *A. stephensi* to support *Plasmodium* sporogonic development to fully infective salivary gland stage sporozoites is dictated by the activities of several known components of the mosquito's innate immune system. In order to increase the yield of sporozoites per mosquito, thereby enhancing PfSPZ manufacturing efficiency, we are genetically modifying mosquitoes to reduce activity of the IMD signaling pathway and the downstream effector molecule LRIM1. Two approaches are being taken: 1) overexpression of CASPAR, a negative regulator of the IMD pathway; and 2) employing novel technologies to reduce expression of the LRIM1 gene by introducing hairpin-DNA transgenic constructs encoding LRIM1-specific siRNAs. We will present our progress generating transgenic lines of *A. stephensi* and the levels of *P. falciparum* sporogonic infection achieved in transgenic mosquitoes relative to appropriate control mosquitoes.

980

WAAL GENE OF *ENTEROBACTER SP.* AG1 FROM THE MOSQUITO *ANOPHELES GAMBIAE*: ROLES IN LIPOPOLYSACCHARIDE (LPS) BIOSYNTHESIS AND OXIDATIVE STRESS DEFENSE

Dong Pei, Wanqin Yu, Jinjin Jiang, Phanidhar Kukutla, Jiannong Xu

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

Enterobacter bacteria are core residents in the gut of mosquito *Anopheles gambiae*, which are dominant in the gut microbial community after blood feeding. We isolated a strain of Enterobacter from the G3 colony of *An. gambiae*, and sequenced its genome. In vitro, the bacterium showed strong tolerance to paraquat, an oxidative stress inducer. In order to identify genes that are involved in the protection against oxidative stress, a mutant library of *Enterobacter sp.* Ag1 was generated using transposon-mediated mutagenesis. One mutant was identified, in which the waaL gene was disrupted. WaaL gene encodes O antigen ligase, which ligates the O antigen to the core-lipid A during LPS synthesis. The mutant had deficient LPS structure on the LPS PAGE gel. The LPS structure was restored in the cells that were complemented with a waaL gene containing plasmid. The mutant grows normally in LB culture with no difference from the wildtype. However, the mutant lost its tolerance to 6mM paraquat. In addition, the mutant was more sensitive to H2O2 stress. Taken together, the data suggest that waaL gene is required for LPS biosynthesis, and is involved in the protection against oxidative stress. LPS is the major component of the cell wall in Gram negative bacteria. The mutant can be used as a model to study the roles of LPS in colonization of the gut and bacteria-host interactions.

981

AEDES AEGYPTI LABORATORY ADAPTATION LEADS TO GLOBAL TRANSCRIPTOMIC DOWN-REGULATION AND DENGUE VECTOR CAPACITY CHANGES

Natapong Jupatanakul, Ana C. Bahia, Shuzhen Sim, George Dimopoulos

W. Harry Feinstone Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Baltimore, MD, United States

Current knowledge of dengue-mosquito interactions is derived from studies that utilized laboratory-adapted mosquito strains maintained in an insectary environment for decades. A better understanding of the genetic and transcriptomic changes that occur in field-derived mosquito strains over the course of laboratory adaptation would provide valuable insight into how mosquitoes adapt to a specific environment and how this adaptation influences vector capacity. After being maintained in a laboratory environment for generations, dengue virus susceptibility of the field-derived mosquito strains changed over time. Microarray analyses of the laboratory-adapted mosquitoes revealed a general down-regulation of the transcript abundance of genes involved in processes such as metabolism, transcription, translation, and immunity, which provides the evidence of a genetic bottleneck due to the laboratory environment. Our result revealed that specific rearing conditions can promote transcript profile changes that may both directly and indirectly affect mosquito susceptibility to dengue virus. Functional assays of these genes provide further insight to the individual contribution of these potential dengue virus host-factors.

982

AEDES MCINTOSHI: GENETIC DIVERSITY AND MAGNITUDE OF RIFT VALLEY FEVER IN KENYA

Poumo David Tchouassi

International Centre of Insect Physiology and Ecology (icipe), Nairobi, Kenya

The complex interplay of virus-host-vector cycle driven by climate change has been attributed to the spread and sporadic outbreak of many arboviral diseases such as Rift Valley fever (RVF). However, the genetics of vectors in conditioning their maintenance and spread is least appreciated despite variation which may be evident in the pattern of outbreak occurrence. We used both mitochondrial and nuclear markers to characterize the genetic structure of *Aedes mcintoshi* populations, a key vector of Rift Valley fever (RVF) virus, from 14 sites including virus-endemic/free areas and epidemic prone areas of Kenya using Neighbor-joining trees, Bayesian inference, median joining network and analysis of molecular variance (AMOVA). Both gene *loci* indicated 4 supported genetic lineages with significant genetic diversity among them across the study areas. The lineages display geographic restriction reflecting the magnitude of RVF in Kenya possibly influenced by prevailing environmental conditions in these locations. Broadly for both markers, lineage I was restricted to Central, Rift Valley and Western areas and lineages III and IV restricted to localities in North Eastern Kenya. However within North Eastern Kenya, the epicenter of RVF epidemics in Kenya, these two lineages (III and IV) occur in sympatry in most of the localities sampled but overall low mean evolutionary divergence estimates between the lineages suggest a variant or cryptic species in that region. Furthermore, disproportionate abundance of these lineages in these localities and their presence/absence may drive differential transmission and outbreak patterns of the disease in different communities of North Eastern Province of Kenya.

983

TIME-VARYING, SEROTYPE-SPECIFIC FORCE OF INFECTION ESTIMATES FOR DENGUE VIRUS USING LONGITUDINAL SEROLOGICAL DATA

Robert C. Reiner, Jr.[1], Steven T. Stoddard[1], Brett M. Forshey[2], Aaron A. King[3], Alicia M. Ellis[4], Alun L. Lloyd[5], Kanya C. Long[6], Claudio Rocha[7], Stalin Vilcarromero[7], Helvio Astete[7], Isabel Bazan[7], Audrey Lenhart[8], Gonzalo M. Vazquez-Prokopec[9], Valerie A. Paz-Soldan[10], Philip J. McCall[11], Uriel Kitron[9], John P. Elder[12], Eric S. Halsey[7], Amy Morrison[1], Tadeusz J. Kochel[13], Thomas W. Scott[1]

[1]University of California Davis, Davis, CA, United States, [2]University of Iowa, Iowa City, IA, United States, [3]University of Michigan, Ann Arbor, Ann Arbor, MI, United States, [4]University of Vermont, Burlington, VT, United States, [5]North Carolina State University, Raleigh, NC, United States, [6]Andrews University, Berrien Springs, MI, United States, [7]U.S. Naval Medical Research Unit - 6, Lima, Peru, [8]Centers for Disease Control and Prevention, Atlanta, GA, United States, [9]Emory University, Atlanta, GA, United States, [10]Tulane University, New Orleans, LA, United States, [11]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [12]San Diego State University, San Diego, CA, United States, [13]Naval Medical Research Center, Silver Spring, MD, United States

The utility of disease models for planning public health interventions and policy relies on accurate estimates of key transmission parameters. These parameters are often estimated as constants in time, not always because of the perceived veracity of that assumption, but often as a consequence of data constraints such as the lack of long-term, longitudinal infection data. One such parameter, the force of infection, is the per-capita risk of a susceptible individual becoming infected. The force of infection captures the fundamental dynamics of transmission and is crucial to gauging necessary control efforts as well as informing vaccine deployment. Dengue virus (DENV) is a multi-serotype, mosquito-borne, viral infection that causes an estimated 390 million human infections each year. Here, we describe a new spline-based fitting procedure developed to compute time-

varying serotype-specific estimates of the force of infection using a 12-year longitudinal DENV dataset from Iquitos, Peru. The dataset contained information on 14,335 individuals (47,121 blood samples) and 23,989 serotype-specific DENV infections. Of these, 3,621 occurred during the study period, which enabled estimation of when the infections took place. Depending on year and serotype, yearly force of infection varied from 0 to 0.33. We identified periods of synchronization between serotypes, but there was no consistent pattern in which serotypes experienced simultaneous outbreaks. As an extension of our approach we calculated time-varying serotype-specific estimates of the basic reproductive number (R0) for DENV, which varied from less than 1 to 5.43, depending on year and serotype. Our results provide important new insights into DENV transmission dynamics that will inform implementation of vector management strategies and deployment of vaccines, when they become available.

984

FINE-SCALE HUMAN MOVEMENT: THEORY, DATA AND IMPLICATIONS FOR DENGUE VIRUS TRANSMISSION

T. Alex Perkins[1], Andres J. Garcia[2], Gonzalo M. Vazquez-Prokopec[3], Donal Bisanzio[3], Robert C. Reiner, Jr.[1], Steven T. Stoddard[4], David L. Smith[5], Thomas W. Scott[4], Andrew J. Tatem[6]

[1]RAPIDD Program, National Institutes of Health, Davis, CA, United States, [2]University of Florida, Gainesville, FL, United States, [3]Emory University, Atlanta, GA, United States, [4]University of California, Davis, CA, United States, [5]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [6]University of Southampton, Southampton, United Kingdom

From traveling on an airplane to commuting for work to visiting neighbors, human movement plays a major role in the spread of infectious diseases at a variety of scales. Models of fine-scale movement (e.g., within a city) and data to inform them have been lacking for resource-poor areas, which are afflicted by a multitude of diseases and where movement patterns are likely to depart substantially from those in developed temperate areas. Moreover, aspects of movement most relevant to the transmission of a mosquito-borne disease such as dengue fever have received little explicit consideration in existing models. We developed a new model for simulating individual human movement and fit it to interview data collected from 149 residents of the city of Iquitos, Peru. We then simulated city-wide movement networks for Iquitos and compared the properties of those networks to movement networks in other settings. Finally, we applied human movements simulated by our model to a recently developed framework for modeling mosquito-borne disease transmission. Our model of individual human movement has five distinct components that together describe variation in how individuals allocate their time across a dynamic set of locations: the number of locations visited, what types of locations are visited, where those locations are, and how often and for how long individuals visit each location. Fitting the model to data revealed that movement differs among locations of different types, that distance from home strongly influences where people go and how often and for how long they visit, and that some variation in movement patterns can be explained by individual attributes, such as age or sex. Network properties of simulated movement across the city differ in several ways from comparable movement networks from cities in developed countries, and they are also sensitive to assumptions about secondary network structures not captured by our model; e.g., social relationships. Combining simulated movements with information about spatial variation in mosquito densities and mosquito movement, we show that disease invasion probabilities and threshold criteria for disease persistence change precipitously as fine-scale variation is homogenized at increasingly aggregated scales.

985

SYMPTOMATIC VERSUS INAPPARENT OUTCOME IN REPEAT DENGUE VIRUS INFECTIONS IS INFLUENCED BY THE TIME INTERVAL BETWEEN INFECTIONS AND STUDY YEAR

Magelda Montoya[1], Lionel Gresh[2], Juan Carlos Mercado[1], Katherine L. Williams[3], Maria José Vargas[1], Gamaliel Gutierrez[2], Guillermina Kuan[4], Aubree Gordon[5], Angel Balmaseda[1], **Eva Harris**[3]

[1]Laboratorio Nacional de Virología, Centro Nacional de Diagnóstico y Referencia, Ministerio de Salud, Managua, Nicaragua, [2]Sustainable Sciences Institute, Managua, Nicaragua, [3]Division of Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States, [4]Centro de Salud Sócrates Flores Vivas, Ministerio de Salud, Managua, Nicaragua, [5]Divisions of Epidemiology and Infectious Diseases and Vaccinology, School of Public Health, University of California Berkeley, Berkeley, CA, United States

Four dengue virus serotypes (DENV1-4) circulate globally, causing more human illness than any other arthropod-borne virus. Dengue can present as a range of clinical manifestations from undifferentiated fever to classic Dengue Fever to severe, life-threatening syndromes. However, most DENV infections are inapparent. Yet, little is known about determinants of inapparent versus symptomatic DENV infection outcome. Here, we analyzed over 2,000 DENV infections from 2004 to 2011 in a prospective pediatric cohort study in Managua, Nicaragua. Symptomatic cases were captured at the study health center, and paired healthy annual samples were examined on a yearly basis using serological methods to identify inapparent DENV infections. Overall, inapparent and symptomatic DENV infections were equally distributed by sex. The mean age of infection was 1.2 years higher for symptomatic DENV infections as compared to inapparent infections. Although inapparent versus symptomatic outcome did not differ by infection number (first, second or third/post-second DENV infections), substantial variation in the proportion of symptomatic DENV infections among all DENV infections was observed across study years. In participants with repeat DENV infections, the time interval between a first inapparent DENV infection and a second inapparent infection was significantly shorter than the interval between a first inapparent and a second symptomatic infection. This difference was not observed in subsequent infections. This result was confirmed using two different serological techniques that measure total anti-DENV antibodies and serotype-specific neutralizing antibodies, respectively. Taken together, these findings show that, in this study, age, study year and time interval between consecutive DENV infections influence inapparent versus symptomatic infection outcome, while sex and infection number had no significant effect. Moreover, these results suggest that the window of cross-protection induced by a first infection with DENV against a second symptomatic infection is approximately 2 years. These findings are important for modeling dengue epidemics and development of vaccines.

986

PHYLOGENETIC EVIDENCE FOR THE EMERGENCE OF A NORTH AMERICAN LINEAGE OF DENGUE VIRUS SUBTYPE 1

Gilberto A. Santiago[1], Ana M. Rivas-Estilla[2], Javier Ramos-Jimenez[2], Ildefonso Fernandez-Salas[2], Jorge L. Muñoz-Jordan[1]

[1]Centers for Disease Control and Prevention, San Juan, PR, United States, [2]Universidad Autónoma de Nuevo León, Monterrey, Mexico

Dengue, one of the most important re-emerging tropical diseases, is caused by four dengue virus subtypes (DENV-1-4) .Transmission in previously non-epidemic areas is documented every year. The proliferation of urban centers, frequent international travel, and considerable climatic changes, probably contributes to the increased distribution of DENV, as evidenced by emergence of new phylogenetic lineages. In 2010, Nuevo Leon, Mexico, a region that had reported only limited dengue cases since 2005, reported 2271 laboratory-positive cases, with 99.6% of the isolates being DENV-1. Concomitantly, Key West, Florida experienced

endemic DENV-1 transmission in 2009 and 2010. These occurrences indicate the establishment of endemic DENV transmission in new areas of North America. We performed in-depth sequence analyses on the envelope gene from 81 DENV-1 isolates from the Americas including 17 sequences obtained from clinical cases during the 2010 Nuevo Leon outbreak and 14 sequences from cases during the 2009-2010 epidemic in Key West. Maximum likelihood and Bayesian phylogenetic analyses identified the emergence of a new DENV-1 lineage currently propagating in North America. This North American lineage emerged from the Central American lineage of the American-African genotype evolving in a short period of time following a northbound transmission trajectory. The recent DENV-1 emergence into non-endemic areas seems to have driven the divergence of new monophyletic sublineages associated with particular epidemics in Nuevo Leon and Key West. Vector density, geographic, and climatic variables were found to be associated with the increase in dengue incidence facilitating the propagation of the mosquito vector and the virus within susceptible populations. *In situ* microevolution of this emerging lineage has been identified confirming the establishment of this lineage in new regions of North America. Our findings reveal the emergence of a new lineage of DENV-1 circulating in North America. The transmission of this virus in new areas seems to have driven the evolution of this lineage. This dynamic of DENV evolution demonstrates the ability of the virus to follow the movement of the human host, and increased transmission in new areas.

987

OUTCOMES OF INFANTS WHOSE MOTHERS HAD SYMPTOMATIC DENGUE INFECTION DURING PREGNANCY: A RETROSPECTIVE COHORT STUDY

Eleanor Friedman[1], Fadi Dallah[2], Emily W. Harville[1], Pierre Buekens[1], Gerard Breart[3], Gabriel Carles[2]

[1]Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States, [2]Franck Joly Hospital, St. Laurent du Maroni, French Guiana, [3]INSERM, Paris, France

Data on dengue infection and its possible effects on the developing fetus are scarce. Existing case reports have suggested higher rates of preterm birth and low birth weight for infants whose mothers had dengue during pregnancy, but many questions remain since, to our knowledge, no epidemiologic investigation on the infant outcomes of mothers with dengue during pregnancy has been done. We conducted a retrospective cohort study in St. Laurent du Maroni, French Guiana, examining the poor birth outcomes of preterm birth (PTB) and low birthweight (LBW) among infants whose mothers had symptomatic dengue during pregnancy. Conditional logistic regression was used to match each of the 86 exposed infants to the three unexposed births immediately following to create a stratum. Due to changes in reporting of miscarriages over time, a sensitivity analysis including and excluding infants at various gestational ages was conducted. The three categories used in the sensitivity analysis were; all infants regardless of gestational age and their strata, only infants 17 weeks of gestational age or older and their strata, and only infants 22 weeks of age or older and their strata. Odds ratios were adjusted for maternal age, maternal ethnicity, maternal gravidity, interpregnancy interval and maternal anemia. After adjustment, PTB demonstrated an increased risk in the dengue exposed group (aOR 22 weeks: 1.41 (0.39, 5.20), aOR 17 weeks: 1.89 (0.61, 5.87), aOR all infants: 3.34 (1.13, 9.89)). Adjusted results for LBW were similar, demonstrating an increased risk in the exposed group (aOR 22 weeks: 1.43 (0.56, 3.70), aOR 17 weeks: 1.67 (0.71, 3.93), aOR All infants: 2.23 (1.01, 4.90)). The results of this study indicate that symptomatic dengue infection in pregnancy may increase the risk of PTB and LBW in infants.

988

COST-EFFECTIVENESS OF A NOVEL TECHNOLOGY FOR DENGUE PREVENTION IN CHILDREN: INSECTICIDE-IMPREGNATED SCHOOL UNIFORMS

Yesim Tozan[1], Pitcha Ratanawong[2], Phanthip Olanratmanee[3], Valerie Louise[2], Peter Dambach[2], Pattamaporn Kittayapong[3], Annelies Wilder-Smith[2]

[1]New York University, New York, NY, United States, [2]Ruprecht-Karls-University Heidelberg, Heidelberg, Germany, [3]Mahidol University at Salaya, Nakhon Pathom, Thailand

Children carry a larger proportion of the disease burden of dengue in endemic countries and are more likely to experience the complications of the disease, including dengue haemorrhagic fever (DHF), compared to adults. Prevention is key to reducing morbidity and mortality because no specific curative treatment exists for the disease. Vector control using adulticides and larvacides has shown some but very limited impact on dengue incidence. The implementation of vector control activities is particularly challenging in urban and peri-urban areas, where the vector is well-established and thriving on proximity to humans. The search for novel, acceptable and affordable methods of vector control is ongoing. Because dengue vectors bite primarily during the day, schools_where children spend most of their day_are an ideal setting for dengue prevention and control. A community-based controlled trial is currently underway in eastern Thailand to assess the impact of impregnated school uniforms on the incidence of dengue in school-aged children. While awaiting the results of the trial, a recent modeling study has shown that "the use of insecticide-impregnated uniforms has an efficacy varying from around 6% in the most pessimistic scenario to 55% in the most optimistic scenarios simulated." Following standard guidelines of economic analyses, we will develop a decision analytical model to evaluate the potential health and economic value of this new intervention from the societal perspective, using data from the published literature specific to Thailand. The model will simulate the incidence of dengue fever and DHF, survival, disability adjusted life years, and costs in a hypothetical cohort of children receiving the intervention and will facilitate a comparison against scenario of no intervention. The outcome of this analysis will be expressed as a ratio of incremental costs to incremental health outcomes of the intervention. We will perform sensitivity analysis of key variables and assumptions.

989

DENGUE-ASSOCIATED HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS IN PUERTO RICO

Esther M. Ellis[1], Emmaculate Lebo[2], Charlotte Baker[2], Janice Pérez-Padilla[1], Tyler Sharp[1], Eduardo Ochoa[3], Hector Díaz-Pinto[3], Luis Clavell[4], Anabel Puig-Ramos[5], Erskin Bezares[6], Liza González[6], Camille Lupiañez-Merly[5], Jorge Muñoz-Jordan[1], Elizabeth Hunsperger[1], Haydee García[7], Francisco Joglar[7], Harold Margolis[1], Kay Tomashek[1]

[1]Centers for Disease Control and Prevention, San Juan, PR, United States, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Puerto Rico Children's Hospital, San Juan, PR, United States, [4]San Jorge Children's Hospital, San Juan, PR, United States, [5]University of Puerto Rico, San Juan, PR, United States, [6]University of Puerto Rico Hospital, San Juan, PR, United States, [7]Puerto Rico Department of Health, San Juan, PR, United States

Hemophagocytic lymphohistiocytosis (HLH) is a rare, potentially fatal disorder characterized by hyperinflammation due to uncontrolled proliferation of activated lymphocytes, resulting in prolonged fever, pancytopenia, jaundice, and hepatosplenomegaly. HLH can be familial or acquired, the latter being the result of malignancy or infection. Dengue-associated HLH (dengue-HLH) has been described in 26 case reports since 1966, but has not been previously recognized in Puerto Rico. In December 2012, CDC Dengue Branch was notified of several dengue-HLH cases at two San Juan pediatric hospitals. An investigation was conducted to: 1)

determine the incidence of HLH since 2008; and 2) determine the infecting agent(s) associated with HLH cases. Medical records were queried to identify patients with findings compatible with HLH. To date, 480 records have been reviewed and 18 patients identified that met accepted criteria for HLH. Sixteen (84%) HLH cases had diagnostic evidence of DENV infection by IgM ELISA (44%) or PCR (56%): dengue virus types -1and -4 were detected. There was one fatal dengue-HLH case (case-fatality rate [CFR]: 5.5%). Dengue-HLH cases ranged in age from 0.2 -16 years, 50% were infants, and all resided in northern Puerto Rico. Among children aged 0-16 years, the annual incidence of dengue-HLH cases was 1.8 per 100,000 population. The median serum ferritin value was 22,524 µg/L (range: 754-522,000 µg/L) in dengue-HLH cases. Hemophagocytosis was evident in bone marrow aspirates of six of 11 (55%) dengue-HLH cases for which testing was performed. Median hospital stay was 26 days (range: 8-81 days). Only one hospital consistently used immunosuppressive therapy for suspected or confirmed HLH cases. We are conducting a case-control study to identify risk factors for developing dengue-HLH and to determine why infants were predominantly affected. Some symptoms of HLH may also be seen in patients with severe dengue, potentially resulting in under-recognition. Physicians in dengue endemic areas should be made aware of HLH.

990

CD47 DEFICIENCY PROTECTS AGAINST *PLASMODIUM BERGHEI* ANKA AND *P. CHABAUDI* AS INFECTION

Kodjo Ayi, W. Conrad Liles, Kevin C. Kain

University of Toronto, Toronto, ON, Canada

Malaria pathogenesis is dependent upon the invasion and maturation of the malaria parasite within host red blood cells (RBCs). Protection against severe and fatal malaria is associated with RBC polymorphisms where structural and functional defects limit invasion and growth of the parasite. CD47 engagement by macrophage SIRPα inhibits phagocytosis and prevents RBC uptake. We have recently shown that CD47 levels are lower on *Plasmodium falciparum*-parasitized RBCs and consequently increases the phagocytosis of parasitized RBCs *in vitro*. In the present study we examine whether blockade of CD47-SIRPα confers protection *in vivo* using two different murine models of severe malaria. C57BL/6 mice lacking CD47 (Cd47-/-) and congenic controls (Cd47+/+) were inoculated intraperitoneally with 106 *P. berghei* ANKA (PbA) or with 106 *P. chabaudi chabaudi* AS (PccAS) parasitized RBCs and were monitored twice daily for 21 days. All congenic control mice infected with PbA died within 6-9 days post-infection with symptoms of experimental cerebral malaria. In contrast, mice with CD47 deficiency displayed profoundly lower parasite burdens ($P < 0.0001$) and had significantly improved survival ($P < 0.0001$) compared with their Cd47+/+ expressing littermates. Similarly, compared to congenic control mice that develop high *parasitemia* when infected with PccAS, Cd47-/- mice were completely refractory to PccAS infection. In conclusion, these results indicate an important role for CD47/SIRPα blockade in protection in murine models of experimental severe anemia and cerebral malaria.

991

T-BET PREVENTS DEVELOPMENT OF PROTECTIVE IMMUNITY BUT MAY SUPPRESS T CELL DEATH INDUCED BY *PLASMODIUM YOELII* 17XNL INFECTION

Miranda S. Oakley, Aditi SenguptaBanerjee, Bikash R. Sahu, Leda Lotspeich-Cole, Victoria Majam, Sheldon L. Morris, Sanjai Kumar

Food and Drug Administration, Bethesda, MD, United States

$CD4^+$ T cells are important mediators of malaria immunity. However, the effect of differentiation programs that lead to the generation of $CD4^+$ T cell subsets and influence other aspects of host immunological networks are poorly understood. We used mice deficient for T-bet, the master regulator of Th1 $CD4^+$ T cell differentiation, to examine the effect of Th1 $CD4^+$ T cells on immune protection to nonlethal murine malaria *Plasmodium yoelii* 17XNL. T-bet-deficient C57BL/6 mice had significantly lower (2.9 fold) *parasitemia* compared to wildtype C57BL/6 mice indicating that the T-bet transcription factor hinders the formation of protective immunity in this murine model. Analysis of the B cell compartment of adaptive immunity demonstrated that T-bet-deficient mice produced abrogated levels of IgG2a and abundant levels of IgG1 antibodies suggesting that altered regulation of antibody isotype switching in the absence of T-bet may be responsible for the enhanced immune protection observed in these mice. Remarkably, absence of T-bet was also associated with a transient but significant loss (2.5 fold) of T cells during the ascending phase of *parasitemia* (day 8) followed by limited expansion of T cells during the descending phase of *parasitemia* (day 14) suggesting that T-bet may suppress malarial antigen induced T cell death. While T-bet-deficient mice had significantly fewer T cells compared to WT mice, there was no observed loss of IFN-γ^+CD4^+ and IFN-γ^+CD8^+ T cells indicating that IFN-γ producing T cells are preferentially expanded in T-bet-deficient mice. These results are further corroborated by a 6.7 fold increase in serum IFN-γ in T-bet-deficient mice during the clearance phase (day 22) of infection. Lastly, T-bet-deficient mice produce comparatively greater numbers of $Foxp3^+CD25^+$ regulatory $CD4^+$ T cells. Future studies are directed at determining whether this excess of $Foxp3^+CD25^+$ regulatory $CD4^+$ T cells is responsible for the early contraction and limited expansion of T cells observed in T-bet-deficient mice and elucidating the mechanism of T-bet mediated suppression of malarial antigen induced T cell death.

992

MAPPING *LOCI* ASSOCIATED WITH PARASITE CLEARANCE AND VIRULENCE USING MURINE MODELS OF MALARIA

Sethu Nair[1], Sittiporn Pattaradilokrat[2], Martine Zilversmit[1], Jian Wu[1], Vijayaraj Nagarajan[3], Wenming Xiao[3], Richard Eastman[1], John Tan[4], Xin-zhuan Su[1]

[1]National Institutes of Health, National Institute of Allergy and Infectious Diseases/Laboratory of Malaria and Vector Research, Rockville, MD, United States, [2]Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand, [3]Bioinformatics and Computational Biosciences, National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [4]The Eck Institute of Global Health, Department of Biological Sciences, University of Notre Dame, Notre Dame, IN, United States

Malaria is the most deadly parasitic disease of mankind, killing more than half a million children each year. A better understanding of the molecular basis governing disease pathology will help reduce mortality and morbidity. Murine malaria parasites have served as important models for studying host-parasite interaction and disease pathogenesis. Here we have genetically crossed two *Plasmodium yoelii* parasites that have different growth characteristics and cause different host mortality, to identify novel parasite factors that can influence host response and disease severity. Fourty-six recombinant progeny were cloned and identified from the cross after typing the progeny with microsatellite markers. Using a new assembly of the *P. yoelii* genome and Next Generation Sequencing data of multiple isolates we generated, we designed a genotyping microarray containing approximately 11,000 single nucleotide polymorphisms between the two parental strains for quick and accurate assessment of progeny genotypes. Quantitative trait *loci* analysis (QTL) using disease phenotypes and genotypes from recombinant progeny identified two chromosomal *loci* on Chromosome 7 and 13 that are linked to parasite growth and disease severity. Further analysis of the *loci* revealed additive interactions between the *loci* identified. We are in the process of validating candidate genes in the *loci* that could potentially be explored for vaccine development.

993

SHIFTED CLEARANCE OF ONCE-INFECTED ERYTHROCYTES CONTRIBUTES TO POST ARTESUNATE NON INFECTIOUS DELAYED ANEMIA (PANDA) IN SEVERE MALARIA PATIENTS CURED WITH INTRAVENOUS ARTESUNATE

Stéphane Jauréguiberry[1], Papa Alioune Ndour[2], Marc Thellier[3], Flavie Ader[4], Sebastien Imbert[4], Oussama Mouri[3], Sylvestre Biligui[5], Liliane Cicéron[5], Sandrine Houzé[6], Eric Kendjo[7], François Bricaire[8], Dominique Mazier[9], Eric Caumes[8], Pierre Buffet[9], French Artesunate Study Group[10]

[1]Infectious Disease Unit, UMRs945 Inserm Paris6, Pitié Salpêtrière Hospital, APHP, Paris, France, [2]UMRs945 Inserm Paris6, Paris, France, [3]CNR Paludisme, Parasitology Mycology Unit, Pitié Salpêtrière Hospital, APHP, Paris, France, [4]Parasitology Mycology Unit, Pitié Salpêtrière Hospital, APHP, Paris, France, [5]CNR Paludisme, UMRs945 Inserm Paris6, Paris, France, [6]CNR Paludisme, Parasitology Mycology Unit, Bichat Hospital, APHP, Paris, France, [7]CNR Paludisme, Paris, France, [8]Infectious Disease Unit, Pitié Salpêtrière Hospital, APHP, Paris, France, [9]Parasitology Mycology Unit, UMRs945 Inserm Paris6, Pitié Salpêtrière Hospital, APHP, Paris, France, [10]Paris, France

Intravenous artesunate is now the recommended first-line therapy for severe malaria worldwide. Recently, an original pattern of anemia that occurs abruptly days to weeks after complete parasite clearance has been reported in European travellers cured by artesunate. The mechanism of this post-treatment complication is unknown. Through an optimized national surveillance program, we collected clinical and laboratory data from 125 *Plasmodium falciparum*-infected travelers treated with iv artesunate. Of 72 patients followed beyond 8 days post-admission (D8), 35 (48%) had a conventional "rising" pattern of hemoglobin concentration without hemolysis after D8, 17 (24%) had a typical abrupt "recurring" anemia pattern Post-Artesunate Non-infectious Delayed Anaemia (PANDA) defined by a greater than 10% drop in hemoglobin concentration and/or rise in LDH concentration occurring after D8, and 15 (21%) had a "persisting", stable anemia/hemolysis pattern. The kinetics of circulating once-infected erythrocytes (from which dead parasites had been removed by the spleen-specific pitting process) was determined in 12 patients. The concentration of once-infected erythrocytes peaked before D8. It was higher than 60% of initial parasitaemia in the 7 patients with subsequent PANDA but lower than 40% of initial *parasitemia* in the 5 patients with other patterns of anemia. During artesunate treatment intra-erythrocytic rings are rapidly killed but host erythrocytes remain intact and return to the circulation after the parasite remnants have been expelled by the spleen-specific pitting process. Pitting clears artesunate-exposed parasites without destroying host erythrocytes but spared once-infected erythrocytes are eventually removed from the circulation 2 to 3 weeks later. This shifted clearance contributes to the peculiar kinetics of recurring anemia in patients cured by artesunate. Early quantification of once-infected erythrocytes may help predict the risk of PANDA in this context.

994

ERYTHROCYTE INVASION RECEPTOR PREFERENCES OF *PLASMODIUM FALCIPARUM* ISOLATES IN GHANAIAN CHILDREN

Henrietta Mensah-Brown[1], Nicholas Amoako[2], James Abugri[1], Godfred Agongo[3], Emmanuel K. Dickson[4], Jose A. Stoute[5], David J. Conway[6], **Gordon A. Awandare**[1]

[1]Infectious Diseases Research Laboratory, Department of Biochemistry, Cell and Molecular Biology, University of Ghana, Legon, Accra, Ghana, [2]Kintampo Health Research Center, Kintampo, Ghana, [3]Navrongo Health Research Center, Navrongo, Ghana, [4]Department of Immunology, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Accra, Ghana, [5]Department of Medicine, Pennsylvania State University College of Medicine, Hershey, PA, United States, [6]Department of Pathogen Molecular Biology, London School of Hygiene and Tropical Medicine, London, United Kingdom

Clinical manifestations of *Plasmodium falciparum* infection are caused by invasion of erythrocytes by the malaria parasite, a process which is mediated by multiple receptor-ligand interactions. Antibodies against some parasite ligands have been shown to significantly inhibit parasite growth *in vitro*, demonstrating that these interactions may be good targets for the development of an effective blood stage vaccine. This study was aimed at investigating the erythrocyte receptors used by *P. falciparum* isolates in Ghana. *P. falciparum* isolates were collected from children aged 2-14 years attending hospitals in three ecologically distinct zones in Ghana: Accra, Kintampo and Navrongo. Erythrocyte invasion assays were performed to test the ability of the parasites to invade erythrocytes treated with neuraminidase, trypsin and chymotrypsin, which selectively remove receptors from the erythrocyte surface. In addition, antibodies against two recently identified receptors, basigin and complement receptor 1 (CR1) were used to determine the dependence of the isolates on these pathways. Two to four assays were performed on each isolate. All 16 field isolates tested so far were capable of invading neuraminidase-treated erythrocytes, with invasion efficiencies of 40-80% relative to untreated erythrocytes, indicating that these parasites had sialic acid-independent invasion phenotypes. Invasion of trypsin or chymotrypsin-treated erythrocytes varied between 20-60% relative to untreated erythrocytes representing the contributions of glycophorins A, B, and C. Furthermore, for nearly all the parasites tested, anti-CR1 antibodies significantly inhibited invasion of neuraminidase-treated erythrocytes, confirming the role of CR1 as the major sialic acid-independent receptor for *P. falciparum*. Additional isolates are being tested and results from about 50 parasites will be presented.

995

IMPAIRED ENDOTHELIAL AND MICROVASCULAR FUNCTION IN *VIVAX* MALARIA IN PROPORTION TO DISEASE SEVERITY

Bridget E. Barber[1], Timothy William[2], Matthew J. Grigg[1], Tsin W. Yeo[1], **Nicholas M. Anstey**[1]

[1]Menzies School of Health Research, Casuarina, Northern Territory, Australia, [2]Queen Elizabeth Hospital, Kota Kinabalu, Malaysia

Plasmodium vivax is now recognised as causing severe disease, including acute lung injury, shock, acute kidney injury and severe anemia. The pathogenesis of disease however is poorly understood. In contrast to *falciparum* malaria, parasite biomass is lower in *vivax* malaria due to a predilection for reticulocytes, and parasite sequestration, the hallmark of severe *falciparum* malaria, does not occur to a significant degree. Endothelial and microvascular function are impaired in severe *falciparum* malaria, with each contributing to impaired organ perfusion, but have not been evaluated in *vivax* malaria. We measured endothelial function using reactive hyperemia-peripheral arterial tonometry (RH-PAT), and microvascular reactivity using thenar muscle near-infrared resonance spectroscopy, in patients with severe (n=8) and non-severe (n=30) *vivax* malaria, and compared results with severe (n=18) and non-severe (n=72)

falciparum malaria and 79 healthy controls (HC). Endothelial function was impaired in proportion to P.*vivax* disease severity (median RH-PAT index: severe *P. vivax* (1.49, IQR 1.37-1.88), non-severe *P. vivax* (1.73, IQR 1.46-2.05) vs HC (1.97, IQR 1.64-2.27; ANOVA p=0.024), with function in severe *vivax* malaria at least as low as in severe *falciparum* malaria (median 1.5, IQR 1.26-1.75). Endothelial function recovered by day 3 in severe *vivax* malaria (2.04, IQR 1.76-2.24; p=0.046). Median microvascular reactivity (StO2 recovery; U/sec) was lower in patients with severe *vivax* malaria (5.44, IQR 3.62-6.01) compared to patients with non-severe *vivax* malaria (6.98, IQR 5.79-7.52; p=0.006) and controls (6.34, IQR 5.21-7.03; p=0.027). Endothelial and microvascular function are impaired in severe *vivax* malaria, with endothelial dysfunction comparable to severe *falciparum* malaria. Endothelial and microvascular dysfunction may underlie pathogenesis of severe *vivax* malaria.

996

DNA SEQUENCE REARRANGEMENTS IN THE *PLASMODIUM VIVAX* GENOME

Ernest R. Chan[1], Didier Menard[2], Arsène Ratsimbasoa[3], Odile Mercereau-Puijalon[4], John Barnwell[5], Peter A. Zimmerman[6], David Serre[1]

[1]Cleveland Clinic Foundation, Cleveland, OH, United States, [2]Institut Pasteur du Cambodge, Phnom Penh, Cambodia, [3]Ministère de la santé, du planning familial et de la protection sociale, Antananarivo, Madagascar, [4]Institut Pasteur, Paris, France, [5]Centers for Disease Control and Prevention, Atlanta, GA, United States, [6]Case Western Reserve University, Cleveland, OH, United States

Plasmodium vivax remains a major public health problem that threatens half of the world's population. The emergence of novel mechanisms of invasion and the rise of drug resistance highlights the need for continued research on this parasite. Recent whole genome sequencing of *P. vivax* from field and monkey-adapted isolates by our lab and others have provided a framework for expanding our knowledge of the molecular polymorphism and global diversity of this parasite. In addition to identifying single nucleotide polymorphisms, whole genome sequencing can also be used to characterize DNA sequence rearrangements. Our analyses reveal very large telomeric deletions, evidence of ectopic recombination in MSP gene clusters as well as deletions and duplications involving well-characterized genes. These rearrangements include the deletion of a gene encoding a Phist protein in the Belem strain, the deletion of the reticulocyte-binding-protein-2-like gene in many strains, and a tandem duplication of ~10kb containing the entire Duffy-binding protein gene in several Malagasy field isolates. The duplication of PvDBP in Malagasy strains is of potential further interest as we, and others have shown that *P. vivax* is capable of infecting red cells and causing malaria in Duffy-negative people. A global survey of *P. vivax* infections revealed that this duplication is common in Madagascar but rare or absent elsewhere. The highly conserved nature of the duplicated sequence suggests this rearrangement occurred in a relatively recent evolutionary time frame. These genomic changes reveal evidence of past or on-going *P. vivax* evolution and suggest avenues by which the parasite can increase its genetic diversity and survival capacity.

997

A NEW MOUSE MODEL FOR FEMALE GENITAL SCHISTOSOMIASIS

Monica Richardson, Chi-Ling Fu, Luke Pennington, Jared Honeycutt, Simon Conti, Justin Odegaard, **Michael Hsieh**

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

Female genital schistosomiasis (FGS) is caused by *Schistosoma haematobium* eggs deposited in the human female reproductive tract by adult *S. haematobium* worms. Although FGS causes pelvic pain, vaginal bleeding, disfigurement, and infertility, it also increases the risks of contracting sexually transmitted diseases such as HIV. The associated mechanisms remain unclear due to the lack of a tractable animal model. To model FGS in mice, we injected *S. haematobium* eggs into the posterior vaginal walls of female mice. This resulted in reproducible, synchronous vaginal granuloma development within 2 weeks post-egg injection and lasting for at least 8 weeks after injection. Flow cytometric analyses of vaginal tissues revealed the presence of T cells with variable expression of the HIV target molecules CXCR4 and CCR5. Granulomata contained CD11b+F4/80+ cells (macrophages and eosinophils) as well as CXCR4+MerTK+ macrophages. Strikingly, vaginal wall-injected mice featured significant urinary frequency despite the egg-injected posterior vagina being anatomically distant from the bladder. We speculate that mammals have evolved a bladder overactivity response to deposition of schistosome eggs in the vagina since egg deposition in the bladder often accompanies FGS. This response would facilitate expulsion of bladder-deposited eggs in order to minimize sequelae from chronic egg-induced bladder inflammation. Ongoing studies will ascertain the biologic basis of this vagina-bladder reflex, as well as characterize the mechanisms by which HIV target cells are recruited to vaginal granulomata.

998

PERSISTENT ORGANOMEGALY ASSOCIATED WITH SCHISTOSOMIASIS AND CORRELATES OF ABNORMAL LIVER PATTERN IN YOUNG KENYAN CHILDREN

Stephanie M. Davis[1], Ryan E. Wiegand[1], Edmund Ireri[2], Fredrick Rawago[3], Fridah Mulama[3], LeAnne Fox[1], Maurice Odiere[3], Kimberly Won[1]

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

Schistosoma mansoni infection is a major cause of liver fibrosis and other morbidity in adults. However, morbidity in young children, currently excluded from mass treatment campaigns, is less defined. We previously reported results of a cross-sectional study, nested within a treatment trial in Mbita, Kenya, of a convenience sample of 201 children under 7 years. *S. mansoni* infection was associated with hepatomegaly and splenomegaly, but not image pattern B (IPB), a liver pattern seen on ultrasound, considered a possible intermediate stage in development of fibrosis due to *S. mansoni*. We now report impact of praziquantel (PZQ) treatment on infection and morbidity in these children, a mean of 3.2 (range 1.6-4.0) months post-treatment and 7 months post-baseline. Data included stool exam for current *S. mansoni* infection, blood tests for malaria by smear and rapid diagnostic test (RDT), and baseline infection status. We also used the WHO Niamey ultrasound protocol to stage hepatosplenic damage from *S. mansoni*, including left-lobe hepatomegaly, splenomegaly and liver image pattern. Longitudinal analyses controlled for age, sex, and RDT result; adjusted cross-sectional models also included clustering by village and current *S. mansoni* infection. Among 137 children with longitudinal organometry data who received PZQ, prevalence of *S. mansoni* infection decreased from 42 to 33%. Heavy-intensity infection (greater than 400 eggs per gram) decreased from 10 to 2%. Malaria prevalence increased from 2 to 17% by smear, and was 36% by RDT. Prevalence of IPB increased from 14 to 21%; hepatomegaly, 54 to 68%; splenomegaly, 30 to 44%. Treatment was not associated with improved organomegaly regardless of initial infection status. Among 146 children with post-treatment organometry, current *S. mansoni* infection was associated with increased hepatomegaly on univariable (prevalence ratio [PR]=1.4; p<.01) and adjusted (adjusted PR [aPR]=1.3; p<.01) analysis but not splenomegaly. Among 180 ultrasounded children, IPB was associated with malaria by RDT on univariable (PR=2.0; p<.01) and adjusted (aPR=2.0, p<.01) analysis but not with current *S. mansoni*. A mean of 3.2 months post-treatment, *S. mansoni* infection was common, and was still associated with hepatomegaly in this young cohort in the setting of interval increases in malaria and organomegaly. IPB may not be pathognomonic for *S. mansoni* infection in young children, but may be caused by malaria.

999

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

Geoffrey Gobert

Queensland Institute of Medical Research, Brisbane, Australia

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

1000

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

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

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

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

1001

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

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

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

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

1002

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

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

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

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